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TUBERCULOSIS IN WILD BUCK LIVING UNDER NATURAL CONDITIONS

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INTRODUCTION.

THE existence of tuberculosis in wild animals living under natural conditions is unique. Actually, the rare occurrence of tuberculosis in sheep is attributed to the fact that this species is kept in an environment which approximates more or less to that enjoyed by wild animals. That the environment itself, however natural it may be, is not alone sufficient to prevent infection by B. tuberculosis will be apparent on perusal of the evidence brought forward in this paper.

Eber (1917), in his exhaustive review on tuberculosis in animals, refers to buffaloes slaughtered at the Budapest Abattoirs as being infected to the extent of 0·18 per cent. These animals, however, were undoubtedly domesticated. Further, Calmette (1923), although he states that "wild animals . . . never contract tuberculosis spontaneously; but, in captivity, they are susceptible to it," yet gives a reference (McCoy and Chapin, 1911) where tuberculosis of bovine type apparently existed in ground squirrels. In the case in question, five animals out of 225 examined at San Francisco for plague showed lesions of the glands, viscera, and lungs. Finally, Fox (1923) adds, "There are no reliable data concerning the existence of Tuberculosis in the wild."

Species Affected.

So far in S. Africa the disease has been observed in Kudu (Strepsiceros strepsiceros) and a Cape Duiker (Sylvicapra grimmi), but some farmers claim to have seen similar cases in Bushbuck on rare occasions. As Kudu have been the commonest subjects brought to our notice, to fully grasp the situation it will be necessary to consider in detail their history and habits.

The Kudu is essentially an African animal, ranging from South Africa to Angola, East Africa, and Abyssinia ; but in former times, according to geological records, it extended far over Europe and Asia. It is, however, important to note that of late years, in the southern Cape Province, they have been confined to the Albany and adjoining Districts.

Andreas Sparrman in 1775, when travelling through the Albany District, noted few Kudu ; Lieut. Paterson, passing over the same area in 1779, apparently saw none, and so with many subsequent observers, until Andries Steedman, in 1835, noted that they were becoming scarce. Fortunately, about 60 years ago, the late Messrs. Knott, Tomlinson, and Buckley, whose properties formed one block in the Koonap area, agreed to rigidly preserve the few remaining Kudu, as otherwise they would probably have been exterminated. From that time they have continued to increase and spread. Finally, by Act 11 of 1900, they were placed under official protection and made Royal Game, an Administrator's permit being necessary to shoot Kudu. Although the number is approximately 2,750 they are seldom seen unless hunted.

The Kudu is one of the finest of antelopes. A full grown bull stands about 4-ft. at the withers, weighs about 400-500 lbs., and has magnificent horns, while the cow is hornless and weighs from 300 to 400 lbs.

A bull is usually accompanied by several cows, and when disturbed, in spite of his huge and wide-spread horns, he is able to travel at a great speed through dense bush, the horns being thrown backwards. Both sexes will take any ordinary 5-ft. fence in a stride, so that they cannot be confined to any particular camp or farm ; but we are inclined to believe that they naturally prefer to remain in one area and only migrate if conditions are adverse. The do not congregate or live in large herds at any time and they sleep under sheltering trees and bushes.

Details Concerning Habitat of Affected Buck.

The country concerned is rough hilly bushveld in the Albany District of the Cape Province (Fig. 1). The altitude is from 1,400 to 2,000 feet, the climate varies from a mild to a hot summer (max. about 105° F.), and tle average rainfall for the past six years has been 13·28 inches. The area is in most parts more or less densely covered with bush, very lightly stocked, and the ground is hard and stony. The vegegation consists almost entirely of shrubs and low trees ranging up to 15-ft. high ; some of the bushes are edible and there is little or

no grass or lowlying herbage, the majority of the grazing being from bushes some distance above the ground. The portion of Albany District carrying Kudu, which is the principal species under consideration, consists of 59 farms (244,730 acres), with 2,841 Kudu, 9,544 cattle, and 31,132 small stock.

FIG. 1.
Bushveld in the Albany District.

The incidence of tuberculosis in the domesticated animals in the above area, as would be expected, is very low, *e.g.*, at the Grahamstown Abattoirs during the past five and half years there have only been two cases (one of which was a cow introduced from the Western Cape Province) out of 17,263 cattle slaughtered. This low incidence may be attributed to the following reasons :—(*a*) the cattle are mostly local grade stock ; (*b*) they are not housed, and (*c*) the area is very lightly stocked. It must be emphasised that some farmers are unwilling to supply information regarding the health of their herds or to have cattle tested unless compensated in the event of reactors being slaughtered. An example, however, of what may be the true position is furnished by the following :— A farmer, who two years ago lost two introduced bulls from what was apparently tuberculosis, agreed to have eighteen cows and heifers tested. Of four which gave a positive reaction two have been destroyed, and in each case lesions were found in the retropharyngeal glands. The two remaining reactors have not yet been slaughtered. The owner in question has

800 cattle running on 4,680 acres of bush country similar to that desdribed above.

Descriptions of Cases Encountered.

On September 2nd, 1927, a Kudu bull's head was received for examination. The parotid, pharyngeal, and submaxillary areas were a mass of enlarged glands the contents of which were purulent. Unfortunately no bacteriological examination was made.

On September 13th, 1927, an adult Kudu cow was shot on farm P. and a post-mortem examination revealed the following: Condition of carcase fair, pharyngeal lymphatic glands enlarged and full of caseous pus; about two-thirds of the entire lung tissue showed very marked pneumonic lesions with caseous centres; advanced lesions were present in the bronchial, mediastinal, portal, and mesenteric lymph glands, while the prescapular and inguinal glands were enlarged but revealed no definite lesions.

Material from a mesenteric lymph gland was placed in a sterile test tube and examined bacteriologically. Smears made from this tissue revealed many acid-fast organisms (indistinguishable from B. tuberculosis) when stained by the Ziehl-Neelsen method. A few contaminating organisms were also present. The remaining material was immediately transferred to a test tube containing 4 per cent. sodium hydroxide to destroy the contaminating organisms. It was placed in an incubator for one hour, centrifugalised, and the sediment inoculated on non-glycerinated blood agar and glycerinated Dorset egg medium. The rest of the sediment was inoculated into two guinea-pigs intraperitoneally and two fowls subcutaneously. Twenty eight days after inoculation several flat granular colonies were noticed on the blood agar. Microscopic examination again showed acid-fast organisms.

One guinea-pig died three weeks after injection and the other one a few months after injection, both with generalised tuberculosis. The fowls remained refractory.

Organisms from blood agar were transferred to non-glycerinated egg medium and after a month's growth 1 c.c. saline suspension of the bacilli was injected into each of three rabbits, one intraperitoneally and two intraveneously. Twenty-one days after injection the rabbits which received the intravenous injections died with lesions in the spleen, liver, and kidneys and massive infection of the lungs. The rabbit which was injected intraperitoneally died after twenty-eight days with marked emaciation and numerous tubercles in liver, serosa of intestines, kidneys, and spleen. The lungs were one mass of tubercles.

On December 9th, 1927, we shot a full grown Kudu bull on the same farm and, as it appears to be extremely difficult to ascertain the age by the dentition, the horn measurements (bull) will be given. In the case in question they measured 47-ins. on the curve, 33-ins. straight from base to tip, 16-ins. apart at the tips, and 10-ins. round the base of the horn. The carcase appeared externally to be in excellent condition, and the only abnormalities present were slight enlargement

of the bronchial and mediastinal glands, and a growth (8⅛-in. by 3½-in.) under the longissimus dorsi muscle and involving the corresponding vertebræ and intercostal lymphatic glands. The intercostal lymph glands were much enlarged and upon incision one of them exposed a dry caseous material.

Guinea-pigs were injected intraperitoneally and subcutaneously with material from this bull. The injection was prepared from a tuberculoma of the spine, adjacent lymph gland, and enlarged bronchial lymph glands. All injected guinea-pigs developed generalised tuberculosis, with ascites in some instances where there was massive infection of liver and lungs.

Cultures were made directly on blood agar from ascitic fluid and infected organs. Tiny colonies of B. tuberculosis were noticed, and acid-fast organisms identified twenty-two days after incubation from both organs and ascitic fluid.

On June 19th, 1928, two Kudu bulls were shot on the same farm, the one being apparently normal, while the other exhibited a large swelling under the left ear (see Fig. 2). Post-mortem examination revealed : Condition of carcase good. Caseation of lymph glands in right and left parotid regions and pharyngx, and consolidated areas in both lungs, some caseous.

FIG. 2.
Kudu bull, showing enlarged parotid lymph gland.

Organs preserved in 50 per cent. glycerine showed the presence of acid-fast organisms on microscopic examination. Guinea-pigs injected

from infected material of lung died twenty-eight days and forty days respectively after injection, with generalised tuberculosis. The lungs in particular were heavily infected. Those injected from bronchial gland were chloroformed to death six weeks afterwards. They also showed the presence of tuberculosis in various organs, but not so extensive as in those injected from lung material.

Cultures were obtained directly from the lesions of these guinea-pigs.

On June 25th, 1928, we shot an adult Kudu cow on farm M.P., but failed to detect any abnormality in the carcase.

On July 7th, 1928, two Kudu cows were shot on farm D.F. One of these, about two years old, was apparently healthy, while the second cow, which was in milk and in excellent condition, showed a swelling about the size of an egg in the left parotid area Further examination of this cow showed enlarged pharyngeal, mediastinal, mesenteric, and bronchial lymph glands, and large consolidated areas in the lungs, and the left prescapular gland was twice the normal size and had a large caseous centre. In the left parotid area the glands were enlarged, and in the right parotid area several glands united to form a caseous mass the size of a turkey's egg.

Bronchial amd mediastinal lymph glands, preserved in 50 per cent. glycerine, showed on microscopic examination the usual acid-fast organisms.

Inoculation of guinea-pigs from these specimens confirmed the presence of tuberculosis.

On July 1st, 1928, a sick adult Duiker ewe, caught in the veld on farm D.F., died at the Institute the same night.

The post-mortem examination of this animal revealed extreme emaciation, miliary tuberculosis of both lungs, pleurisy, generalised tubercular peritonitis, nodules in the kidney and liver tissue, and diseased mediastinal, bronchial, portal, and prescapular lymph glands. Tubercular lesions were also present in the mesenteric, crural, precrural, popliteal, and pharyngeal glands.

The lungs and glands and a portion of the peritoneum were preserved in 50 per cent. glycerine.

The lungs and pleura were studded with numerous greyish-white tubercles. The glands were enlarged and hard. The peritoneum displayed numerous greyish-yellow miliary tubercles.

Scrapings from cut surface of lung stained by the Ziehl-Neelsen method revealed numerous acid-fast organisms.

Cultures were made on blood agar and glycerinated Dorset egg medium. Three weeks after inoculation tiny flat colonies were seen on blood agar medium and microscopically B. tuberculosis was observed. Two guinea-pigs and two rabbits were injected intraperitoneally with 1 c.c. from broth in which a piece of lung tissue had been shaken.

Thirty-seven days after injection one rabbit died with extensive generalised tuberculosis, showing numerous tubercles in the lungs, liver, peritoneum, and serosa of intestines. The second died fifty-eight days after injection with similar lesions. The guinea-pigs,

killed fifty-six days after injection, showed a massive infection of all internal organs.

Microscopic Appearances.

The microscopic pathology of these cases is typical of bovine tuberculosis. In each instance there were central necrosis, epithelioid cells, and many Langhans' giant cells (see Fig. 3).

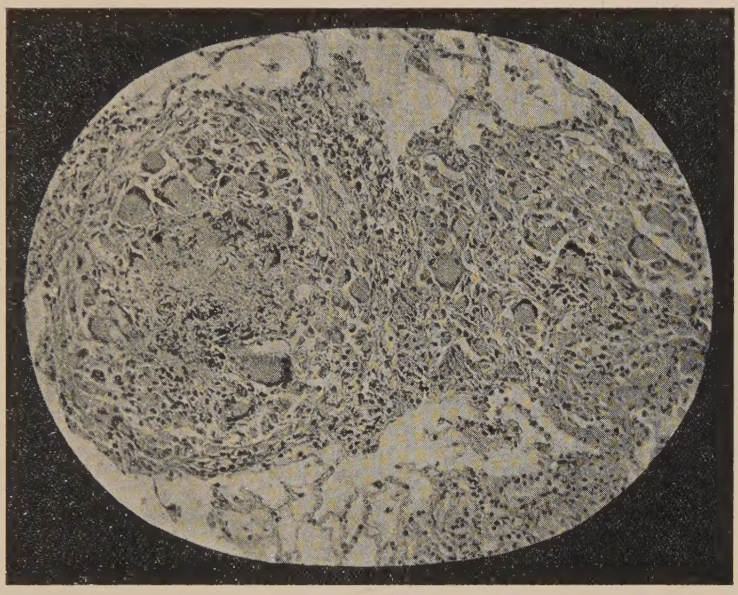

FIG. 3.
Section of Duikerlung (× 115). Shows early stage of caseation, with numerous giant and epithelioid cells.

Probable Method of Infection.

From the habits of wild buck one feels that ingestion must be the main method of infection, and yet the nature of the grazing tends to minimise this source. It has been suggested, and it appears feasible, that, during the recent record drought, the dams and water holes were either dry or very low, and that game or stock drinking from such a limited supply contaminated the water and so caused the disease to spread.

In any case, it appears as though the disease has found a most susceptible and favourable subject in wild buck in spite of the environment.

Other Wild Game in the Albany District.

Large numbers of Springbuck are preserved on several farms adjoining, but not in the Kudu area, and until recently one farm carried about 80 Blesbuck, but these died during the late drought. There is no evidence to show that tuberculosis existed amongst the

Blesbuck, nor have cases yet been detected in other local wild animals such as Stembuck, Rheebuck, Wild Pig, Ostrich, Meercat, Guinea-fowl, or Jackal.

Public Health Aspect.

In concluding we must bear in mind one serious aspect in this connection, and that is the fact that a considerable amount of Biltong (dried meat) is made from raw buck flesh.

Conclusions.

The strains isolated so far from Kudus and a Duiker may be summed up as highly pathogenic for rabbits and guinea-pigs. The few fowls tested were refractory. The isolated organisms are dysgonic and non-glycerophilic, especially in the first generation. We have little doubt from the above findings that the organisms studied are strains of B. tuberculosis bovis.

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EXPERIMENTAL OBSERVATIONS ON ANTHRAX INFECTION AND ANTI-ANTHRAX IMMUNISATION.

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Introduction.

Besredka's Principle of Local Immunisation.

The classical method of anti-anthrax immunisation by the subcutaneous injection of anthrax vaccines can be carried out successfully in the case of larger susceptible animals, but it has been found difficult and often fails absolutely in small laboratory animals such as rabbits and guinea-pigs. Besredka (1921) has drawn attention to the manner in which the skin reacts to Pasteur's anthrax vaccines, and has succeeded in immunising rabbits and guinea-pigs by cutaneous inoculation of attenuated cultures, followed by virulent anthrax bacilli. He states that the immunity produced in guinea-pigs by such a procedure is due

not to the presence of antibodies in the blood, but to a local reaction in the skin, and that the serum from an animal immunised in this manner does not protect another against the subcutaneous injection of the second vaccine or a small quantity of virulent bacilli.

Besredka is of the opinion that since some particularly vulnerable portal of entry is essential for infection, protection against invasion can only be obtained by the local immunisation of the surface tissue which constitutes the specific portal of entry, irrespective of any generalised reaction of the other cells of the body as evidenced by the presence of antibodies in the serum. Further, he affirms that when such a specific area is immunised the body as a whole is protected against subsequent infection. Following this principle he has directed his attention to the experimental production of immunity against various infections by the vaccination of the tissue at the portal of entry of the disease in question. In this way he has found it possible to produce a local immunity of the intestine against paratyphoid bacilli by the oral administration of the living organism along with ox bile ; of the respiratory tract against B. diphtheriæ by the intratracheal injection of killed cultures of the organism ; and of the skin against B. anthracis by the intracutaneous injection of anthrax vaccines. Should his views be confirmed it would indicate that in future attempts to create artificial immunity should be carried out by the local vaccination of particular tissues. The experiments recorded in this paper have been undertaken in order to test this theory of local immunity in the case of anthrax infection.

Cutaneous Infection by Bacillus Anthracis.

Besredka believes that in the guinea-pig the anthrax bacillus has a particular affinity for the skin in which it multiplies and secretes its toxin, and that in other tissues the organism is harmless. This has been confirmed by Balteanu (1922), who found that animals injected subcutaneously, intravenously, or intraperitoneally with virulent anthrax bacilli did not die if precautions were taken to avoid injury and infection of the skin at the time of inoculation.

The following experiment was undertaken to test this claim. Two rabbits were inoculated intravenously with an emulsion of B. anthracis through the marginal vein of the ear. In one animal the site of inoculation was cauterised thoroughly by the application of a red-hot solder bolt to prevent infection of the skin ; the other rabbit served as a control. Both rabbits died within three days. On autopsy there was very little noticeable to the naked eye apart from congestion of the internal organs ; gelatinous œdema was present only in a small area around the site of inoculation in the ear of the control animal. The organism was recovered from the heart-blood and spleen of each animal.

The experiment was repeated and a minimal amount of inoculum used but the animals died as before.

Beltrami and Romat (1923) obtained similar results, but Besredka believes that this was due to the insufficient cauterisation of the skin ;

he points out that after cauterisation of the ear the organism has ample time to return to the point of inoculation and so infect the skin. It should be noted, however, that there was no evidence in the experiments recorded above of any inflammatory œdema of the subcutaneous tissue at the site of inoculation in those rabbits in which cauterisation of the ear had been carried out, as would be expected had infection of the skin occurred.

The importance of the skin in anthrax infection has also been noted by Plotz (1924), who demonstrated that laboratory animals survived the subcutaneous injection of anthrax bacilli if care were taken not to infect the skin at the time of inoculation. The method he employed was to insert 1 to 3 c.c. of a culture of virulent bacilli in capsules of collodion or glass or in capillary tubes in the subcutaneous tissue, and after the skin had completely cicatrised to liberate the organisms by breaking the capsules. Beltrami and Romat (1923) were unable to obtain such results in similar experiments ; Besredka holds that the failure of their experiments was due to insufficient cicatrisation of the skin.

Experimental Infection by Other Routes.

That infection does not take place through the conjunctival sac has been demonstrated by Aitoff (1922), who was unable to infect laboratory animals by the instillation into the conjunctiva of a dense emulsion of bacilli from agar cultures. None of the animals showed the slightest reaction although the organism was found in the conjunctival sac on the following day and in some cases even after seven days had elapsed.

It has also been shown by Besredka (1920) and by Brocq-Rousseu and Urbain (1924) that very large numbers of anthrax bacilli can be injected intratracheally in animals without producing ill effects if precautions are taken to avoid infecting the skin.

Boquet (1924) points out that infection following the ingestion of B. anthracis does not occur as long as the mucosa of the alimentary tract remains intact, but that infection takes place when the organism is injected into the tongue. It is of interest to note that Pasteur was able to infect animals by the oral administration of anthrax bacilli only when some sharp material such as bristles, which injured the mucosa, were mixed with the food. Sobernheim (1913) states that the ingestion of spores is responsible for the infection of cattle through the intestine, but that considerable numbers of them are necessary for infection to take place. More recently Sanarelli (1924) has shown that while the vegetative forms of anthrax bacilli are destroyed by the gastric juice of rabbits and guinea-pigs, spores may invade the blood either from the intestine or the lungs and may remain ingested by phagocytes for a number of days without injuring the animal. If, however, the resistance of the animal is lowered by increased temperature or by the injection of a cytolytic substance, such as lactic acid, which destroys phagocytes without harming the organism, the liberated spores rapidly germinate and produce a fatal septicæmia. This work

has been confirmed by Basset (1925), who found that anthrax spores were much less pathogenic than vegetative cultures of the bacilli.

Another interesting observation was made by Boquet. He obtained positive blood cultures from six out of ten guinea-pigs which had previously ingested anthrax bacilli, but he found that after bleeding the animals by cardiac puncture they all died of a typical anthrax infection. This he considered was due to the traumatism of the skin since the characteristic œdema was found at the point where the needle penetrated the skin. Control animals that were not bled survived. Boquet also showed that guinea-pigs which had ingested bacilli died of anthrax if the skin was injured by shaving, irritation, or contusion, whereas control animals in which the skin was intact survived.

The following experiment confirms to a certain extent these observations. Six guinea-pigs were fed daily with bran in which an emulsion of anthrax bacilli was mixed. After ten days two of the animals died while the rest remained apparently healthy. On autopsy only slight pathological changes were noted, but the organism was isolated in pure culture from the heart-blood in each case. The surviving guinea-pigs were still fed with anthrax bacilli and a week later a small quantity of blood was withdrawn from each animal by cardiac puncture and inoculated into tubes of bouillon. Before carrying out this procedure the hair of the chest wall of the guinea-pig was removed by depilation and the surface of the skin sterilised with spirit and iodine, and the animals were placed in fresh cages to avoid any extraneous contamination. The cultures in each case yielded growths of B. anthracis. Each of these guinea-pigs died within four days after cardiac puncture. On autopsy two of them showed pathological lesions typical of an anthrax infection. The characteristic œdema was present in the subcutaneous tissues, but it was not localised or specially marked at the point where the needle penetrated the skin during the heart puncture. The other two animals showed no obvious lesions.

This experiment is of great interest and would indicate that, as the result of the ingestion by guinea-pigs of sporing cultures of anthrax bacilli, the organisms reach the blood stream and may circulate in the blood without producing definite pathological effects. According to Sanarelli (1924), they are carried in the spore phase by phagocytes, and an analogous phenomenon has been recognised in the case of the tetanus bacillus by Vaillard and Vincent (1891-92), Francis (1914), and others. The death of the guinea-pigs after heart-puncture seems, therefore, to be more likely due to some factor related to this experimental procedure, possibly traumatic in nature, which favours the rapid germination of the spores present in the circulating blood, rather than to the contamination and infection of the subcutaneous tissues by the withdrawal of the needle used in the course of heart puncture.

Many other workers deny that the skin is the only organ sensitive to anthrax and have been able to produce lethal effects in animals by injecting the organism into various situations in the body even when precautions were taken not to infect the skin. It has been shown by

Cernaianu and Suhatzeanu (1924) that rabbits do not survive the intravenous or intraperitoneal injection of the organism or inoculation into the brain, liver, or muscles, even when precautions are taken to avoid infecting the skin. On autopsy bacilli were obtained from the blood of such animals but they were unable to find any œdema of the skin or subcutaneous tissue, indicating that contamination of these tissues had not occurred at the time of inoculation. Combiesco (1923) confirms the work of Besredka and Balteanu but makes the reservation that the inoculation subcutaneously, intravenously, or intraperitoneally of anthrax bacilli does not produce the disease in rabbits or guinea-pigs as long as the quantity injected does not exceed a certain limit. He affirms that when the dose injected is very large the animals die in spite of all precautions to avoid infecting the skin. Combiesco doubts if the skin is really a sensitive organ and thinks that it has a purely mechanical action, and that in this situation phagocytosis occurs more slowly than in other parts of the body and in this way allows the bacilli to become encapsulated. He states also that phagocytosis of the organism does not take place when they have formed capsules, and that the death of an animal after the injection of a large number of bacilli is due to the inability of the leucocytes to ingest all the organisms before capsule formation occurs. Similar results were obtained by Gratia (1924), who found that the intravenous injection of anthrax blood containing capsulated bacilli killed rabbits even although careful precautions were taken to avoid infecting the skin.

Wollman (1925) is of the opinion that the ease with which capsule formation takes place in the skin does not by itself explain the importance of skin infection. He states that the number of bacilli deposited in the skin when contamination takes place after an intraperitoneal or intravenous injection is very much smaller than a minimal lethal dose of the organism and is therefore incapable of causing death. He attributes the fatal infection in such cases to the combined action of the extra-cutaneous organisms and those deposited in the skin. Wollman thinks that the action of contamination of the skin in the process of anthrax infection is probably due to the abundant production, in the area of the skin infected, of substances favouring infection (Bail's aggressins), and that the superiority of cuti-vaccination over other methods of immunisation might be due in part to the particularly favourable conditions that it creates for the production or absorption of such substances and in consequence to the establishing of an anti-aggressin immunity.

Immunisation of Laboratory Animals by Cutaneous Inoculation of Anthrax Vaccines.

As the result of his theory that infection takes place only through the skin, Besredka believes that immunity to anthrax can only occur as the result of cutaneous immunisation. He has succeeded in immunising guinea-pigs and rabbits either by applying vaccines to a scarified area of skin or by injecting them intracutaneously. Plotz found that rabbits vaccinated by the intracutaneous method obtained

such a high degree of resistance that they withstood the injection of five hundred times the lethal dose of B. anthracis, whereas those vaccinated subcutaneously obtained only a very slight immunity or none at all. Similarly, Brocq-Rousseu and Urbain (1924) have demonstrated that guinea-pigs which survived the intratracheal injection of large doses of anthrax bacilli when subsequently inoculated intracutaneously or subcutaneously did not withstand even a minimal lethal dose of the organism. They found, however, that animals vaccinated by the cutaneous method survived the injection into the lungs, liver, kidneys, or peritoneal cavity of very virulent anthrax bacilli, indicating that a solid immunity had been acquired by the previous vaccination. In addition, it has been shown by Besredka and Trevise (1922) that immunised guinea-pigs withstand the inoculation of anthrax blood as well as cultures of virulent bacilli.

It should be noted that other methods of vaccination have been successful. Cordier (1925) was unable to vaccinate animals successfully by the intracutaneous method, but succeeded when the vaccines were injected intratesticularly or into the medullary cavities in bones. Gratia (1924) found " rough " and " smooth " types of colonies of B. anthracis and was able to immunise rabbits successfully by the intravenous injection of the " smooth " type followed later by the " rough " type. That cutaneous immunisation is successful in larger animals has been shown by Brocq-Rousseu and Urbain (1923), who immunised horses by this method. They were unable to find any agglutination, deviation of complement, or precipitation reactions with serum obtained from immunised horses, and found that such immune serum did not protect guinea-pigs from small doses of the second anthrax vaccine. Monod and Velu (1925) have recorded 21,640 intradermal vaccinations which were carried out by veterinary workers in Morocco. Of these, 14,405 were performed on cattle, 2,520 on sheep, 4,640 on pigs, and 75 on horses, and in each case vaccination was carried out with one single intracutaneous injection. The results obtained were very satisfactory, as the animals vaccinated were exposed without harm in a badly infected area.

Guinea-Pigs.

In view of the results obtained by the authors quoted above, it is of interest to record the following experiments in laboratory animals. The vaccines of B. anthracis which were used had been attenuated by Pasteur's method and were obtained from Professor A. Besredka ; the technique employed was as described in his original paper.

Six guinea-pigs were inoculated cutaneously by shaving an area of skin on the abdomen, 2 by 4 inches, so that the surface appeared raw and red, and a swab soaked in the " first vaccine " was rubbed thoroughly over the scarified area. In 24 hours the inoculated surface was intensely red and showed dark necrotic patches which coalesced together, so that in 48 hours a necrotic " scab " had formed over the abdomen. The inflammatory reaction was limited to the site of inoculation and in two days' time the redness was not so marked and the

slough was starting to separate from the abdominal wall. In the next four days the skin gradually returned to normal, but three of the guinea-pigs appeared very thin and wasted. The " second vaccine " was applied by scarification of the healed area in a similar manner to that described above. The inflammatory reaction which was present in 24 hours was more severe than after the " first vaccine," two of the guinea-pigs dying in 48 hours, and the rest four days after the application of the " second vaccine." Control animals inoculated with the " second vaccine " only died in 48 hours. On autopsy the animals that died in 48 hours and the control animals showed the typical pathological lesions of an anthrax infection. In the other animals the subcutaneous tissue showed only slight inflammatory changes, and there was no gelatinous œdema present. Congestion of the internal organs was present and was most marked in the small intestine, especially in the control animals, which also showed a sero-sanguineous exudate in the peritoneal cavity. Bacilli were numerous in films of heart-blood and in spleen smears, and the McFadyean reaction was well marked. The organism was recovered on culture of the heart-blood of each animal.

Since the method of inoculation used in the above experiment did not permit an exact estimate of the amount of vaccine administered, a further series of six guinea-pigs were inoculated intracutaneously with 1·0 c.c. of the " first vaccine." Following the injection there was some swelling, redness, and induration at the site of inoculation in each animal, but the local reaction was not severe and there was no necrosis of the skin. Three of the guinea-pigs died in four days' time and showed typical pathological changes on autopsy. The surviving guinea-pigs were inoculated intracutaneously with 0·2 c.c. of the " second vaccine " but all died within three days and showed similar post-mortem findings.

Another series of six guinea-pigs was inoculated, starting with smaller quantities, but although they survived the intracutaneous injection of 0·25 c.c. and 0·5 c.c. of the " first vaccine " they all died after receiving 0·1 c.c. of the " second vaccine."

The results detailed above indicate that the cutaneous method of immunising guinea-pigs with anthrax vaccines may be quite unsuccessful, and that, although many survived the " first vaccine," all the animals died when only small quantities of the " second vaccine " were introduced into the skin.

Rabbits.

The cutaneous immunisation of rabbits although not entirely successful has given more satisfactory results. Out of eight rabbits inoculated in this manner six survived while two died during the process of immunisation, the first four days after receiving 1·0 c.c. of a 48 hours' broth culture of virulent bacilli, and the second three days after the injection of the " second vaccine." A possible explanation of the death of these animals is that a small quantity of inoculum may have been injected into the subcutaneous tissue as well as into the

skin, although care was taken to avoid this occurring when the rabbits were inoculated.

The surviving rabbits, with one exception, received numerous injections of virulent anthrax bacilli without any ill effects. The detailed histories of these animals are as follows :—

Rabbit 1.

18/11/27.	0·2 c.c. " Vaccine I " injected intracutaneously.
20/11/27.	Slight inflammatory reaction at site of inoculation with some induration of skin.
22/11/27.	Skin normal.
23/11/27.	1·0 c.c. " Vaccine I " injected intracutaneously.
25/11/27.	Only slight inflammatory reaction of skin, no induration.
27/11/27.	Skin normal.
28/11/27.	0·2 c.c. " Vaccine II " injected intracutaneously.
30/11/27.	Slight inflammation and induration of skin.
2/12/27.	Skin normal.
3/12/27.	1·0 c.c. " Vaccine II " injected intracutaneously.
4/12/27.	Swelling, redness and induration of skin.
9/12/28.	Skin normal.
10/12/28.	0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
12/ 1/28.	1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously. Localised reaction at site of inoculation in ear ; redness, swelling, and œdema.
27/ 1/28.	Ear normal.
12/ 2/28.	2·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously and 2·0 c.c. intraperitoneally .
5/ 3/28.	1·0 c.c. B. anthracis 48 hours' broth culture injected intraperitoneally.
23/ 3/28.	1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously.
2/ 4/28.	1·0 c.c. anthracis 48 hours' broth culture injected intraperitoneally.
2/ 5/28.	1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 1·0 c.c. intraperitoneally.
12/ 5/28.	1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 1·0 c.c. intraperitoneally.
18/ 5/28.	0·5 c.c. agar slope emulsion (opacity No. 6 Brown's standard) injected intravenously and 0·5 c.c. injected intraperitoneally.
24/ 5/28.	Rabbit died.

On autopsy there was no gelatinous œdema of the subcutaneous tissues, and apart from some congestion of the spleen and intestine there was no obvious pathological lesion. The organism was recovered in large numbers from the spleen and heart blood. It should be noted that on May 24th, 1928, the same quantity of an emulsion of anthrax bacilli was injected into Rabbit 7, but in spite of having withstood a greater number of injections of B. anthracis Rabbit 1, which had previously appeared quite healthy, died. The explanation of the death of this animal probably lies in the fact that the immunity produced by this method of immunisation, while sufficient to protect it from moderate doses of B. anthracis, does not avail against an excessively large dose of the organism.

Rabbit 2.

25/1/28. 1·0 c.c. " Vaccine I " injected intracutaneously.
27/1/28. Swelling and redness with some induration at site of inoculation.
1/2/28. Skin normal. 0·5 c.c. " Vaccine II " injected intracutaneously.
3/2/28. Redness and induration of skin.
5/2/28. Skin normal.
10/2/28. 1·0 c.c. " Vaccine II " injected intracutaneously.
12/2/28. Inflammatory reaction at site of inoculation.
14/2/28. Skin normal.
15/2/28. 0·25 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.
18/2/28. Slight inflammtory reaction at site of inoculation.
25/2/28. Skin normal.
5/3/28. 1·0 c.c. 48 hours' broth culture B. anthracis injected intracutaneously.
7/3/28. Only very slight inflammatory reaction at site of inoculation.
7/3/28. Rabbit died.

On autopsy localised gelatinous œdema was present in subcutaneous tissues at site of inoculation. Internal organs deeply congested and B. anthracis was recovered from the local lesion, spleen, and heart-blood.

Rabbit 3.

25/1/28. 1·0 c.c. " Vaccine I " injected intracutaneously.
27/1/28. Swelling, redness, and induration of site of inoculation.
28/1/28. Skin at site of inoculation becoming necrotic.
5/2/28. Necrosis still present.
7/2/28. Necrotic scab separating from skin.
10/2/28. Skin practically normal. 0·5 c.c. " Vaccine II " injected intracutaneously.
12/2/28. Redness and induration of skin at site of inoculation.
14/2/28. Skin normal.
15/2/28. 1·0 c.c. " Vaccine II " injected intracutaneously.
18/2/28. Slight inflammatory reaction at site of inoculation.
5/3/28. 0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
14/3/28. 0·5 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
23/3/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
29/3/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
11/4/28. 2·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
2/5/28. 2·0 c.c. B. anthracis 48 hours' broth culture injected intraperitoneally.
12/5/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 2·0 c.c. injected intraperitoneally.
21/5/28. Rabbit exsanguinated by heart puncture and serum preserved.

Rabbit 4.

6/2/28. 1·0 c.c. " Vaccine I " inoculated intracutaneously.
8/2/28. Slight redness and induration of skin at site of inoculation. No necrosis.

10/2/28. Skin normal. 0·5 c.c. " Vaccine II " injected intracutaneously.
12/2/28. Slight redness and induration of skin at site of inoculation.
14/2/28. Skin normal.
15/2/28. 1·0 c.c. " Vaccine II " injected intracutaneously.
18/2/28. Rabbit died. Result of autopsy similar to Rabbit 2.

Rabbits 5 *and* 6.
11/2/28. 1·0 c.c. " Vaccine I " injected intracutaneously.
12/2/28. Redness and induration of skin at site of inoculation.
14/2/28. Skin normal.
15/2/28. 0·5 c.c. " Vaccine II."
18/2/28. Redness and induration of skin.
25/2/28. Skin normal.
5/3/28. 1·0 c.c. " Vaccine II " injected intracutaneously.
14/3/28. 0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
23/3/28. 0·5 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
29/3/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
2/4/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
11/4/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
2/5/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
12/5/28. 2·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
21/5/28. Rabbits exsanguinated by heart puncture and serum preserved.

Rabbit 7.
10/2/28 to 23/3/28. Intracutaneous injections of " Vaccines I and II."
29/3/28. 0·25 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
2/4/28. 0·5 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
11/4/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
18/4/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
2/5/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intracutaneously.
12/5/28. 1·0 c.c. B. anthracis 48 hours' broth culture injected intravenously and 2·0 c.c. intraperitoneally.
18/5/28. 0·5 c.c. of an emulsion of a 24 hours' agar slope culture of B. anthracis (opacity No. 6 Brown's standard) injected intravenously and 0·5 c.c. injected intraperitoneally.
24/5/28. 1·0 c.c. emulsion from agar slope culture (opacity No. 8 Brown's standard) injected intravenously.
31/5/28. Rabbit exsanguinated by heart puncture and serum preserved.

Rabbit 8.
10/2/28 to 23/3/28. Intracutaneous injections of " Vaccines I and II."
28/3/28. 0·25 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.

2 4/28. 0·5 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.
11/4/28. 1·0 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.
20/4/28. 1·0 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.
2/5/28. 1·0 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.
7/5/28. 1·0 c.c. of a 48 hours' broth culture of a virulent strain of B. anthracis injected intracutaneously.
11/5/28. Rabbit exsanguinated by heart puncture and serum preserved.

Immunisation of Rabbits by Subcutaneous Injection of Anthrax Vaccines.

It has been found impossible to immunise rabbits by the subcutaneous injection of anthrax vaccines as is shown in the following experiments :—

Rabbit 9.
10/2/28. 1·0 c.c. " Vaccine I " injected subcutaneously.
12/2/28. Localised swelling at site of inoculation.
14/2/28. Rabbit normal.
17/2/28. 0·5 c.c. " Vaccine II " injected subcutaneously.
19/2/28. Rabbit died. Post-mortem findings typical of anthrax infection

Rabbit 10.
10/2/28. 1·0 c.c. " Vaccine I " injected subcutaneously.
12/2/28. Localised swelling at site of inoculation.
14/2/28. Rabbit normal.
17/2/28. 0·5 c.c. " Vaccine II " injected subcutaneously.
19/2/28. Localised swelling.
23/2/28. Normal.
25/2/28. 1·0 c.c. " Vaccine II " injected subcutaneously.
26/2/28. Rabbit died. Post-mortem findings typical.

Four other rabbits were treated in this manner, three of which died after the injection of 0·5 c.c. of the " second vaccine " while the fourth animal died after the injection of 1·0 c.c. of this vaccine, as in the case of Rabbit 10.

Experiments with the Sera of Immune Animals.

The mechanism of the production of immunity after cutaneous vaccination presents many difficulties. Besredka believes that the skin is the only organ that is sensitive to anthrax infection, and that as soon as this tissue is protected the body as a whole is immune. He states that the serum of an animal cutaneously immunised has no protective properties, and when injected into a guinea-pig along with a lethal dose of B. anthracis does not prevent the death of the animal. This has been confirmed by Brocq-Rousseu and Urbain (1923), who found that such serum as well as having no protective properties did not contain any complement-deviating antibodies, agglutinins, or precipitins. On the other hand, Gratia (1924), found agglutinins in the serum of immunised animals active in a dilution of 1 : 10,000, and states that protective substances can be demonstrated when the serum is injected *along with* the bacilli in guinea-pigs and not separately as in the experiments of Besredka.

Protective experiments were carried out with serum of rabbits immunised solely by the cutaneous method, and with the serum of Rabbit 7 which had received injections of anthrax bacilli intravenously and intraperitoneally as well as by the cutaneous route. The method employed was to inject a fixed quantity of serum (1·0 c.c.) along with a fixed quantity (0·5 c.c.) of decimal dilutions of an emulsion of B. anthracis into mice.

With each experiment a control series of mice were inoculated with 0·5 c.c. of different dilutions of B. anthracis only.

Injection of Graduated Doses of an Emulsion of B. anthracis (opacity corresponding to No. 8 Brown's standard) in Mice along with Serum of Rabbit 8 *(cutaneously immunised).*

	B. anthracis Emulsion only.	*B. anthracis Emulsion and 1·0 c.c. Serum.*
(1)	0·5 c.c. emulsion diluted 1 : 1000 Died 30/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 30/5/28.
(2)	0·5 c.c. emulsion diluted 1 : 10,000 Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 31/5/28.
(3)	0·5 c.c. emulsion diluted 1 : 100,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum Died 30/5/28.
(4)	0·5 c.c. emulsion diluted 1 : 1,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 31/5/28.
(5)	0·5 c.c. emulsion diluted 1 : 10,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 31/5/28.
(6)	0·5 c.c. emulsion diluted 1 : 100,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 1/6/28.
(7)	0·5 c.c. emulsion diluted 1 : 1,000,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 31/5/28.
(8)	0·5 c.c. emulsion diluted 1 : 10,000,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 1/6/28.
(9)	0·5 c.c. emulsion diluted 1 : 100,000,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 1/6/28.
(10)	0·5 c.c. emulsion diluted 1 : 1,000,000,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 31/5/28.
(11)	0·5 c.c. emulsion diluted 1 : 10,000,000,000,000. Died 31/5/28.	0·5 c.c. emulsion plus 1·0 c.c. serum. Died 1/6/28.
(12)	Serum control 1·0 c.c. serum only. — Survived.	

None of the mice which received the serum outlived the corresponding control animal for any length of time and on autopsy all showed pathological changes typical of anthrax infection.

The experiment detailed above was repeated with the serum of Rabbit 7, which had been immunised by intravenous and intraperitoneal injections as well as cutaneously, and also with Sclavo's anti-anthrax serum, with similar negative results in each case.

Discussion.

Anti-anthrax immunity presents problems of great complexity. In the first place we still lack definite knowledge regarding the offensive mechanism of the organism. No toxic substances have been demonstrated in culture, yet anthrax is a disease with undoubted toxæmic manifestations. It has, therefore, been assumed that the anthrax baccillus only produces its specific toxin when growing in the tissues. Bail (1910) considered this to be analogous to aggressin and claimed that the action of anti-anthrax serum is due to anti-aggressins, *i.e.*, an antitoxic agent. There is no evidence that anti-anthrax immunity is due to anti-bacterial substances and Sobernheim (1910) and others have failed to detect any bactericidal action in immune sera. Sclavo's serum has been found to contain a precipitin, as evidenced by Ascoli's reaction, but this is certainly not a protective anti-substance.

Besredka believes that a local cutaneous immunity is the essential feature of resistance to anthrax and maintains that this can be brought about by cutaneous vaccination of animals with anthrax vaccines. Such a method produces an immunity of the skin, and as, according to his view, the other tissues are naturally resistant the animal thus acquires a general immunity.

The manner in which such local immunity is produced has not, however, been satisfactorily explained. Besredka has developed the following theory : There is a marked susceptibility of the skin, in which a " reaction takes place between the receptive cells of the reticulo-endothelial layer and the anthrax bacillus " ; the virulent bacillus has a great affinity for these receptive cells, and this is followed by a " reaction which liberates a new substance, a product of secretion or disintegration of the organism " ; when this substance is present in sufficient quantity it produces a negative chemotaxis which repels the leucocytes and the bacillus is free to multiply ; if a slightly virulent organism is employed, such as the " first vaccine," possessing only a feeble affinity for the receptive cells, the new product which he has postulated appears only in small quantities insufficient to repel the leucocytes, and phagocytosis takes place. He explains in this manner the mechanism of acquired immunity and considers that cutaneous vaccination by diminishing the initial sensitiveness of the receptive cells allows them to become adapted to the organism, so that a fresh infection of virulent bacilli fails to produce a negative chemotaxis and the bacilli are readily removed by phagocytosis.

He states also that the survival of animals after the intraperitoneal or intravenous injection of anthrax bacilli, without contaminating

the skin, is due to the complete phagocytosis of the bacilli, which in the absence of negative chemotaxis are eliminated like inert foreign particles.

Other observers hold that skin susceptibility to anthrax is not specific, and have shown that any area with relatively slight blood supply, such as the brain, testicle, or muscle, is just as susceptible as the skin. In such areas there is a relative absence of protective cells and fluids which allows the organism to modify and adapt itself either by capsule or aggressin formation and so invade the rest of the body. With this view the writer is inclined to agree.

There seems little doubt that the cutaneous immunisation of animals with anthrax vaccines as advocated by Besredka gives successful results, especially when applied to the larger susceptible animals, and that the immunity produced is superior to that obtained by subcutaneous inoculation Further, cutaneous vaccination has proved a safer method of inmunisation since fatalities are much less frequent than when the vaccines are administered by the subcutaneous route.

In the series of experiments on guinea-pigs recorded in this section it was found impossible to immunise these animals either by intracutaneous injections or by the application of vaccines to a scarified area of skin. This appears to be due to their extreme susceptibility since they do not survive even when the vaccines are administered in very small quantities. The method has been more successful in rabbits, which attained a remarkable degree of immunity as evidenced by their tolerance of numerous injections of virulent anthrax bacilli, but the immunity produced, even in rabbits, cannot be regarded as absolute since one animal died after the intravenous injection of a massive dose of the organism. The experimental infection produced in this rabbit was, however, many times greater than would occur under natural conditions. By analogy, sheep and cattle immunised in this way would be adequately protected against epizootic anthrax. On the other hand, none of the rabbits which were being immunised by the subcutaneous route survived the inoculation of the vaccines, and in this way it can be said that cutaneous immunisation offers a method that is more likely to be successful in animals which are markedly susceptible to the disease.

Anti-anthrax serum has been employed therapeutically for some years, and has been found to have preventive and curative properties, which, however, cannot be attributed to the presence of any demonstrable antibodies. It has been shown above that neither commercial anti-anthrax serum nor serum obtained from rabbits immunised by different methods protect mice from injections of very small quantities of anthrax bacilli. This may be due to the extreme susceptibility of mice to the infection, as in the case of guinea-pigs, and such negative results with these animals may not be of definite significance. It should be noted that mice and guinea-pigs, as contrasted with man and the less susceptible animals, do not possess in the slightest degree any natural immunity, and it is possible that the therapeutic action of Sclavo's serum may depend on a reinforcement of a natural immunity mechanism. In the absence of such natural immunity the serum

would exert no protective properties. In this connection, however, the observations of Kraus and Beltrami (1921) are of special importance. They have claimed that normal ox serum has equal effects with immune anthrax serum in experimental anthrax in rabbits, which could not be due to any protective substances since the ox itself is naturally highly susceptible to the disease. The effect of the serum might therefore be entirely non-specific and depend on a non-specific reaction due to the injection of an alien protein.

It seems probable that the skin is not only one of the most susceptible of tissues to anthrax infection, but is also highly sensitive to an immunising stimulus. If immunity depends on the production of a new immune substance or the augmentation of a pre-existing immune substance, it might be supposed that this principle is most readily elaborated by the skin, and that cutaneous vaccination supplies the optimum immunising stimulus.

Two agencies must undoubtedly contribute to anti-anthrax immunity—phagocytosis and some adjuvant principle which acts by inhibiting or neutralising the toxic bacterial products responsible for injury to the phagocytic cells or for the negative chemotaxis which has been postulated as a factor in successful infection. As suggested, immunity (natural or acquired) must depend also on some principle which promotes phagocytosis. Such principles have been long designated opsonins, and both natural and immune opsonins have been demonstrated in relation to phagocytosis by polymorph leucocytes. The relationship of such principles to other phagocytes is more obscure, but it is obvious that toxic bacteria must exert a considerable resistance against their destruction by phagocytic body cells, whereas detoxicated organisms are easily phagocytosed like inert foreign particles.

In the case of an organism which produces a diffusible toxin in culture media, such as the diphtheria bacillus, antitoxin can be readily demonstrated by experimental methods. The existence of an antitoxic principle for the anthrax bacillus is not, however, so readily susceptible of experimental proof for the reasons discussed above, but must remain at present hypothetical. It might be suggested, however, that anti-anthrax immunity is dependent on such agency, and by postulating such substance as a free principle in the blood serum the therapeutic action of Sclavo's serum might be explained. Natural resistance of animals to whatever degree it is manifested may be a function of this prinicple, occurring as a natural immune substance, and in this connection it is of great interest that a partially resistant animal may be more readily immunised that one of the highest grade of susceptibility. It is well known that horses possessing natural antitoxin respond more readily to immunisation with diphtheria toxin than animals without natural antitoxin (Glenny, 1925). Before such questions can be fully answered further analyses are required of the protective action of anti-serum particularly in animals of moderate natural resistance. The writer's own experiments have failed to demonstrate such effect in animals of high susceptibility. It is possible, of course, that in such animals

susceptibility depends on an intrinsic deficiency of the phagocytic system which cannot be influenced by any opsonic or antitoxic agents, and in such case even a powerful opsonic or antitoxic serum would be ineffective. It is also noteworthy in regard to this question that it has been impossible to immunise actively highly susceptible animals by cutaneous vaccination.

Until the exact manner in which the anthrax bacillus produces its pathogenic effect in the body is more clearly understood, the mechanisn of the production of immunity to the disease must remain doubtful. Besredka's conception of the action of the organism on the skin is of particular interest in relation to this problem and his method of local immunisation is of undoubted practical value in the prophylaxis of anthrax. Moreover, his work on the general question of local immunity has opened up a new field of immunological study the exploration of which may lead to new principles and conceptions of resistance to disease.

Summary and Conclusions.

(1) A " solid " immunity to anthrax can be produced by cutaneous vaccination of animals of moderate susceptibility (rabbits), cultures attenuated by Pasteur's method being employed for the purpose.

(*b*) Cutaneous immunisation is attended by less risk of fatal results than other methods.

(*c*) The serum of these animals does not confer protection against the most minute doses of infection in highly susceptible animals (mice).

(2) It has not been possible to immunise animals of high susceptibility (guinea-pigs) by this method ; these animals have invariably succumbed to either the " first " or " second vaccine " (after survival from the " first ").

(3) It seems doubtful whether Besredka's claim holds good that the skin is the only tissue susceptible to B. anthracis.

(4) Anthrax spores may be introduced into the blood stream through the alimentary mucosa and remain latent in the blood for a certain length of time, only germinating and producing an active infection as a result of some additional factor.

(5) The question of local immunity and the question of anti-anthrax immunity are discussed with particular reference to cutaneous vaccination.

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NASAL GRANULOMA OF CATTLE IN BIHAR AND ORISSA.

Experiments regarding mode of Infection and Treatment.

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I.—Introduction.

Nasal Granuloma appears to have existed amongst the working bullocks in certain parts of this Province for a very long time. The disease is believed to be commonly prevalent in other provinces of India too. Its occurrence in the Madras Presidency was first reported by two veterinary assistant surgeons in 1919 in the third issue of the "Madras Veterinary Journal," while its existence in Assam was mentioned in an official communication of the Muktesar Research Laboratory. Mention of this disease does not occur in English or Continental literature, so that it would appear to be confined to the Indian peninsula. The disease generally goes by the name of "Nakhra" or "Sinsunia," *i.e.*, an affection of the nostrils. The popular idea

is that the disease is an incurable one, there being considerable depreciation in the value of an affected animal owing to the difficulty of breathing interfering with work capacity.

Messrs. Krishnamurti of the Madras Veterinary College and H. Cooper of the Muktesar Institute studied the disease, particularly in relation to the pathological condition of the growth on the schneiderian membrane and the causal organism responsible for the disease. The former read a very interesting article on the subject and demonstrated some specimens of the disease in the second Veterinary Conference held at Calcutta in 1923. Both these authors described a ray fungus as the causal organism, akin to the kind which is responsible for actinomycosis ; but the exact mode of infection, the biological character of the organism, and the period of incubation are still obscure. All forms of medical and surgical treatment had proved valuless until 1921, when the veterinary surgeon of Saidapet Veterinary Hospital reported having used tartar emetic with conspicuous success, and was even led to regard the drug as a specific for the disease, although Mr. Krishnamurti was inclined to consider that it had no curative value. The drug has since been used with considerable success in the treatment of a number of cases and it has even been reported that a veterinary assistant in Madras cured an advanced case with a single massive dose of tartar emetic administered intravenously. The drug would thus appear to possess an undoubted curative value, although the statement that a single injection of the drug is capable of effecting a cure in the case of chronic affection without any detrimental results being produced in the patient needs confirmation.

II.—Occurrence of Nasal Granuloma in Bihar and Orissa and Application of Tartar Emetic in the Field.

In January, 1923, and again in March of the same year, specimens of Nasal Granuloma were forwarded to the Imperial Institute of Veterinary Research, Muktesar, and detailed reports on pathological condition of the growths were obtained. Six affected cattle were also despatched to the Institute during August, 1923, in order that they might be subjected to careful investigation.

During the cold weather of 1924 the Director, Civil Veterinary Department, Bihar and Orissa, decided to have an enquiry made into the incidence of the disease in the Province, and the Veterinary Inspectors of the Central Range were instructed to report the number of affected animals in their respective jurisdictions and to test the curative value of intravenous injection of tartar emetic whenever an opportunity for the application of the drug presented itself. The number of cases reported was 1,513, all appearing in an enzootic form, and, out of these, 71 cases were treated with intravenous injection of tartar emetic in doses ranging from 10 to 20 grains in 20 c.c. of distilled water. Treatment could not be carried out in the case of the others.

In one out of six villages in which the operation was conducted the animals were treated by means of five injections each, in four others two to three injections, whilst in the sixth village not more than

one injection was given to each animal. The cases treated with the maximum number of injections were actually either cured or at any rate improved in condition, but those treated with fewer doses did not appear to have derived any lasting benefit. The number of deaths reported was only five. Although the immediate results of the treatment would thus appear to have been satisfactory, the actual curative value of the drug had to be determined by the permanence of the therapeutic effect produced in the treated animals.

III.—Transmission of Nasal Granuloma and Treatment of the Disease under Experimental Conditions.

With a view to studying the method of infection and to ascertain the value of the treatment as expressed by the permanence of the therapeutic effect produced by it, a series of experiments was conducted upon five healthy and eight diseased cattle. The experiments extended over a period of nine months, from March 28th to December 23rd, 1924.

All the experimental animals were maintained as far as possible under the same conditions as obtain in the villages, and were in addition allowed to graze together during the period from July 1st to November 14th, 1924. During the progress of the experiments all variations in weather conditions except severe cold were experienced.

(*A*) *Mode of Infection.*

Ordinarily, the disease is supposed to be directly transmitted from infected to healthy animals, but the possibility has also been suggested of the causal agent being harboured in saprophytic form or otherwise in soil, fodder, or water.

In order to test these possibilities a series of experiments was performed upon a herd of animals maintained at the Bankipur veterinary hospital, composed as follows :—

Nos.	1	to	6	affected.
,,	7	to	10	unaffected.
,,			11	affected (returned as unsuitable).
,,			12	unaffected.
,,			13	affected.

The following is a brief account of the experiments carried out :—

1.—*Feeding and Watering Healthy and Diseased Animals in the same Trough.*

(*a*) No. 7, aged Taylor breed cow, weight four maunds and 30 seers, purchased in the city. Fed and watered with :—

(i) No. 1 bullock (affected with acute disease) from March 28th to July 12th, 1924.

(ii) No. 5 bullock, from July 13th to September 12th, 1924.

(iii) No. 13 bullock, from September 13th to December 21st, 1924.

Result.—The test animal failed to contract the infection.

(*b*) No. 8 Bull calf, 1½ year old, weight 1 maund and 30 seers, purchased in the city. Fed and watered with :—

No. 5 (affected with subacute disease) from March 27th to September 13th, 1924.

As it failed to take the infection, a further attempt to set up the disease was made on September 15th, 1924, by means of an intravenous injection of 5 c.c. of emulsion of morbid material obtained from No. 13.

Result.—The test animal failed to contract the disease.

(*c*) No. 12, aged bullock of medium size, brought from north bank of the Ganges (Hazipur). Fed and watered with :—

(i) No. 6 bullock, from September 5th to 11th, 1924.
(ii) No. 13 bullock, from September 12th to 14th, 1924.
(iii) No. 6 from September 15th to December 21st, 1924.

Allowed to graze with the diseased animals on the maidan.

Result.—The test animal failed to contract the disease.

2.—*Rubbing on the Scarified Surface of Healthy Nasal MucousMembrane Material from a Diseased Nasal Growth.*

(*a*) *Healthy Bullocks Nos.* 9 *and* 10.—Aged, of medium size. The former weighed 4 maunds and the latter 4½ maunds, both being brought from Hazipur (north bank of the Ganges). On July 13th, 1924, the left nasal mucous membrane of bullock No. 10 was well scarified and rubbed with material obtained from a fresh diseased nasal growth in an out-patient (Case No. 550 of the Bankipur veterinary hospital). The patient represented a typical case of nasal granuloma and was brought from a village about eight miles from the city.

The material was ground in a mortar to the consistency of thick paste and was then thoroughly rubbed into the scarified healthy mucous membrane.

The same operation was repeated on September 15th, 1924, with fresh material obtained from No. 13, also a typical case of nasal granuloma. The material was obtained by scraping the affected mucous membrane with a scoop and collecting it in a Petri dish.

Operations repeated after six days.

Result.—The test animal failed to contract the disease.

(*b*) On November 12th, 1924, another specimen of diseased growth obtained from an untreated case of typical nasal granuloma from village Nargoda in Dinapur Sub-division, and the material rubbed on the scarified mucous membranes of the healthy animals Nos. 7 and 12.

Result.—The test animal failed to contract the disease.

3.—*Subcutaneous Injection on both sides of Nasal Peak of Healthy Animal with an Emulsion of Nasal Growth.*

On September 15th, 1924, fresh nasal growth obtained from case No. 13 was ground down in normal salt solution and 30 c.c. of the emulsion was injected into healthy bullock No. 12 under the skin on both sides of the nasal peak.

Result.—The test animal failed to contract the disease.

4.—*Submucous Injection of Healthy Nasal Mucous Membrane with Infected Material.*

On November12th, 1924, about 2 c.c. of an emulsion made from the fresh material from an untreated typical case of nasal granuloma obtained from village Nargoda (Dinapur Sub-division) was injected under the mucous membrane of the left nostril of healthy bullocks Nos. 9 and 10, admitted on July 12th, 1924.

Result :—The test animal failed to contract the disease.

5.—*Intravenous Injection of healthy Animal with Infected Material.*

On September 15th, 1924, a thin emulsion in normal saline solution was prepared from the material obtained from case No. 13, and 5 c.c. of this was injected intravenously into healthy bull calf No. 8, admitted on March 27th, 1924.

Result :—The test animal failed to contract the disease.

6.—*Application of "Nose Strings" used on Diseased Animals.*

On August 9th, 1924, two nose strings used on two typical cases were obtained from the Khajpura village, about four miles from the Veterinary Hospital, and were inserted, while still moist, into the nose of healthy animals Nos. 9 and 10.

The nose strings after insertion were occasionally pulled up and down until the nostrils bled. This process was continued for about a week.

On August 25th, 1924, the nose string of No. 11 diseased animal was interchanged with that of No. 9 healthy bullock and the process of pulling it up and down was continued for three days.

Result.—The test animal failed to contract the disease.

(*B*) *Curative Treatment with Potassium Iodide and Tartar Emetic.*

Treatment was applied in the case of seven diseased animals as follows :—

1.—*Intravenous Injection of Potassium Iodide at Intervals of Seven Days.*

(*a*) No. 1, an aged bullock, weight 5½ maunds, bought at Bhagbatipur, about eight miles from the hospital.

When admitted it snored even while resting and showed symptoms of severe asphyxiation when it was made to work. An examination of the nostrils revealed the presence of ulcerating growths which bled on the slightest manipulation. The animal was believed to have been affected for about six months.

(*b*) No. 6, an aged bullock, purchased from the same village ; weight 6½ maunds, condition fair.

Snoring was not marked, but examination of nostrils revealed ulcerating growths.

Treatment.—The drug was dissolved in distilled water in the proportion of 10 grains to an ounce, and administered intravenously, at intervals of seven days, at the dose of 10 grains in the case of No. 1, and 5 grains in the case of No. 6. The treatment commenced on

March 28th, and terminated on July 9th, 1924, so that No. 1 received a total of 140 grains and No. 6 70 grains of potassium iodide.

Result.—No improvement up to July 13th, 1924.

2.—*Intravenous Injection of Tartar Emetic at Intervals of Two Days.*

No. 5, a six-year-old bullock of medium size ; weight 6 maunds, purchased from the village Bhagbatipur on March 27th, 1924, and admitted to the hospital the same day.

It showed thick ulcerating granular growths in both nostrils, markedly narrowing the nasal passages, with a thick whitish discharge. The condit on of the animal was fair.

Treatment.—The animal received 170 grains of tartar emetic in 34 injections at the above intervals between March 28th and July 12th, 1924, the dose used being 5 grains of tartar emetic in 30 c.c. of distilled water.

Result :—The animal showed no symptoms of reaction to the drug. It was cured and discharged on October 20th, 1924, and no recurrence of the disease was reported up to the date of the compilation of this paper.

3.—*Intravenous Injection of Tartar Emetic at Intervals of seven Days.*

No. 3, a grey bullock, five years old, of medium size ; weight 4 maunds and 10 seers, purchased from the same village, and admitted to the hospital on March 27th, 1924.

This animal was suffering from subacute type of the disease. Snoring not very marked, growth not prominent. The condition of the animal was fair.

Treatment.—140 grains of tartar emetic were given in 14 injections at intervals of seven days, the dose used being 10 grains of tartar emetic in 20 c.c. of distilled water.

Result.—The animal showed no symptoms of reaction to the drug. It was cured, and signs of recurrence were not noticed up to September 22nd, 1924, when it was sold to the Patna Administration Committee. No recurrence of the disease has been reported up to the date of the compilation of the paper.

4.—*Intravenous Injection of Tartar Emetic at Intervals of Seven Days.*

No. 2, a brown, aged bullock, of medium size ; weight 6 maunds and 10 seers, purchased from the same village and admitted on March 27th, 1924.

This animal was known to have been suffering from the disease for about two years. Abnormal breathing was very marked on admission.

Treatment.—It received 108 grains of tartar emetic in nine injections up to July 1st, 1924, at the rate of 12 grains of tartar emetic in 30 c.c. of distilled water at intervals of eleven days. The treatment was discontinued after the ninth injection on July 1st, 1924, by which date all the visible symptoms of the disease had disappeared completely. On September 1st, 1924, *i.e.*, two months after the last injection,

it was noticed that the nasal mucous membrane had become slightly rough and superficially ulcerated. The animal was kept under observation up to October 3rd, 1924, and no further aggravation of the symptoms was reported. As the administration of two additional doses at an interval of four days was not followed by any marked improvement, the drug was given every alternate day, in doses of 10 grains dissolved in 20 c.c. of distilled water. In this manner it received 90 grains of the drug in nine doses up to December 4th, 1924, when it was discharged as cured.

5.—*Intravenous Injection of Tartar Emetic at Intervals of Fourteen Days.*

No. 4, an aged bullock of medium size, weight 4 maunds ; purchased along with other diseased animals from the same village and admitted on March 27th, 1924.

The animal appeared to have been suffering from a subacute form of nasal granuloma, although the duration of the disease could not be ascertained.

Treatment.—The animal was given 80 grains of tartar emetic in eight injections at intervals of 14 days from March 28th to July 11th, 1924. There was no recurrence of the growth up to October 3rd, 1924, when it was sold to the Patna Administration Committee.

6.—*Treatment with Potassium Iodide followed by Tartar Emetic.*

Case No. 1, which received 140 grains of potassium iodide, having shown no improvement, was given 15½ grains of tartar emetic in 50 c.c. of distilled water intravenously. The animal died the same night.

Case No. 6, which received 70 grains of potassium iodide in 14 injections, having shown no improvement, was given 105½ grains of tartar emetic in 10 injections, the first dose being 15½ grains in 50 c.c. of distilled water and subsequent doses 10 grains each, at intervals of 7 days. The animal showed all signs of recovery after the last injection.

Case No. 13. This animal, which was admitted on 12th September, 1924, was used as a control and it also provided material for experiments with effect from 30th November, 1924 ; it was treated by means of tartar emetic administered in 10 grain doses, in 20 c.c. of distilled water, at 4 days intervals. It had thus received 90 grains of the drug when it was discharged as cured. A large hard and painful swelling was noticed after the last injection. It later developed into an abscess, but it eventually healed without giving much trouble.

Summary of Results.

(*a*) *Experiments on Transmission of Nasal Granuloma.*

(1) Three clean cattle were fed and watered in company of diseased animals.

(2) Two clean cattle had their scarified mucous membrane rubbed at different times with material obtained from fresh nasal growths, the material being derived from three different sources.

(3) One bullock was injected subcutaneously on both sides of the nasal peak with 30 c.c. of an emulsion prepared from a nasal growth.

(4) Two clean cattle were given submucous injections of an emulsion made from an untreated case of typical nasal granuloma.

(5) One healthy bull calf was given an intravenous injection of an emulsion prepared from diseased material.

(6) Two healthy bullocks (Nos. 9 and 10) had strings from diseased animals put on their noses and the strings were occasionally pulled up and down until the nostrils bled.

The test animals in all cases failed to contract infection.

(*b*) *Treatment with Potassium Iodide and Tartar Emetic.*

Altogether seven cattle were treated and these resolve themselves into three categories :—

(1) Cases Nos. 1 and 6 were first injected with 5 and 10 grains doses of potassium iodide intravenously at intervals of seven days. The first animal received 140 grains and the second 80 grains of the drug in 104 days, but neither showed improvement. Both the animals were then given tartar emetic intravenously. Case No. 1 died after receiving the first injection of 15½ grains in 50 c.c. of distilled water. Case No. 6 was discharged as cured after 105½ grains of tartar emetic were given in ten injections, the first injection being of 15½ grains of tartar emetic in 50 c.c. of distilled water.

(2) Cases Nos. 2, 3, 4, and 5 were regularly treated with varying doses of tartar emetic at different intervals. The first animal (Case No. 2) received 108 grains of tartar emetic in nine injections and subsequently received 90 grains in nine injections. No. 3 received 140 grains of tartar emetic in 14 injections. No. 4 received 80 grains in eight injections, and No. 5, 170 grains in 34 injections. The best results were observed in the case of No. 4, which made a complete recovery after it had received only a few injections.

(3) Case No. 13 was first used as a control, but was afterwards treated with intravenous injections of tartar emetic administered in 10 grain doses at intervals of four days. The animal recovered after receiving 90 grains.

Conclusions.

Experience in the field and the results of the experiments described above indicate that under ordinary conditions nasal granuloma cannot be transmitted from animal to animal or through the agencies of soil, fodder, or water.

Apparently the infection also cannot be transmitted by subcutaneous, submucous, or intravenous injection of diseased material.

The disease also does not appear to be capable of transmission through the nose string of affected animals ; at any rate the experiment failed to demonstrate such a possibility.

Treatment with potassium iodide is not effective.

For a medium-sized animal the best results may be expected from injections of 10 grains of tartar emetic in 20 c.c. of distilled water administered at intervals of four days.

Massive doses do not appear to have any curative effect. A sound plan would appear to be to repeat the injections in small doses (10 to 12 grains) and at less than four days intervals. When smaller doses are employed the interval may be reduced to one day.

The administration of the drug by the subcutaneous route cannot be recommended, as such a procedure results in the formation of large and painful local swellings lasting for many days.

Note.—Dr. J. T. Edwards, Director of the Imperial Institute of Veterinary Research, Muktesar, in commenting upon this article, has made some valuable observations regarding treatment with potassium iodide and tartar emetic which are quoted below for general information. I believe this suggestion for the use of tartar emetic will prove to be the most practical as well as the safest method of treatment for adoption in the field.

" It is curious to note that potassium iodide exerts no beneficial effect upon the condition, especially as this drug has a pronounced effect upon the clinically similar condition, actinomycosis.

" It may be that this is evidence that the condition is not in reality a streptothricosis, in spite of the fact that ' granules ' apparently indistinguishable from those of actinomycosis are discoverable in the lesions.

" I note that one of your bullocks succumbed after the administration of 15½ grains of tartar emetic in 50 c.c. of water intravenously, and that the dose you recommend varies from 10 to 12 grains in 20 c.c. of water.

" We have found here (Muktesar) that cattle tolerate doses of 5 c.c. of a 3 per cent. solution per 100 lbs. body weight quite readily, although when the blood is rich in trypanosomes the administration of this dosage may be followed by accidents due, it seems, to sudden destruction of the trypanosomes and occlusion of the capillaries. For the large animals in your experiments, averaging 500 lbs body weight, our dosage would therefore be 0·75 gram (or about 12 grains), which is just about the rate you recommend for treatment. We have found in addition that this dosage can be given daily for a number of days (10 or perhaps more). It is not safe to increase this dosage, although some animals will tolerate twice the rate (*i.e.*, 10 c.c. per 100 lbs.) for three days.

" In distributing the drug for treatment I think it would be wise to put it up in freshly prepared solutions containing a certain percentage of tartar emetic (say 3 per cent.), and instruct your staff to give it at a certain rate per unit body weight ; otherwise you may get accidents from overdosage.

" Also, I believe that in this treatment there is no question of a *dosis sterilisans magna*, *i.e.*, sterilisation by the administration of a single dose, but rather a therapeutic effect caused by the cumulative action of the drug administered in suitably large doses repeated at suitable intervals. We know that tartar emetic is excreted, or, at least, its action is not discernible in the blood stream (surra experiments) after an interval of three days. I see no reason, therefore, why the drug should not be administered at very short intervals, with the object of curtailing (if the end results are dependent upon

the total amount of the drug administered) the period of treatment. Again, according to this view, the initial doses should be small, to test the idosyncrasy or the susceptibility of the animal to the drug.

" It is of interest to compare the mode of treatment adopted in human schistosomiasis (bilharziasis) with tartar emetic (see Byam and Archibald, the Practice of Medicine in the Tropics, Vol. III). If an analogous system were adopted for a bullock of 500 lbs. body weight the system would be :—

" (A) First day, 2 grains ; second day, 4 grains ; third day, 6 grains ; fourth day, 8 grains ; fifth day, 12 grains.

" (If preceding dosages are tolerated : sixth, seventh, and eighth days same dosages as on the fifth.)

" (B) Allow one clear week to elapse without treatment and then repeat the fifth day treatment for three successive days.

" (C) Send the bullock now away, to be returned for inspection in a month's time, and, if the lesions have not disappeared, repeat the treatment given under B."

A DETERMINATION OF THE NON-SPECIFIC AGGLUTININ CONTENT OF NORMAL BOVINE SERA, AND ITS BEARING ON THE INTERPRETATION OF THE AGGLUTINATION TEST FOR BOVINE CONTAGIOUS ABORTION.*

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The agglutination test for contagious abortion in cattle has been the subject of much investigation in most countries where the disease is prevalent, but, although it has been largely used in recent years as a reliable means of diagnosis, no general standardisation of technique has taken place, such as has been urged by Seddon[1], Fitch[2], and other workers.

The lowest titre of serum which can be considered to give a specific diagnostic reaction has never been generally agreed upon, and laboratory workers are much at variance in their opinions on this point.

It is unnecessary to record the various standards adhered to. Suffice it to say that although some workers consider that water-clear agglutination at a dilution of serum of 1 in 25 is evidence of infection, others are not prepared to express a definite opinion on reactions obtained with a lower dilution than 1 in 100.

M'Fadyean and Stockman[3] were the first to draw attention to the fact that the serum from a normal cow possessed the power of agglutinating B. abortus in low dilutions, and they were not at that time (1909) able to place the test on a satisfactory basis.†

By 1912 they[4] had pursued their investigations far enough to bring the test beyond the probationary stage.

* This article was found among Mr. Holtum's papers after his lamented death in September last.

† See this Journal, 1928, p. 364.

The sera from 535 *presumably* healthy cattle, mainly steers, were tested, in order to throw more light on the limit of agglutinating power possessed by non-infected animals. Sera from 526 of these failed to show any distinct agglutination at 1 in 50. Nine showed " some agglutination " at that dilution. One steer showed complete agglutination at 1 in 200, and later on at 1 in 500. The serum of a calf showed partial agglutination at 1 in 200, but it was not complete at 1 in 50. The sera of two bulls agglutinated completely at 1 in 50.

" In the great majority of these cases the blood was taken while the animals were being killed at a slaughter-house, and naturally in such cases their past history was not obtainable."

As all the cattle were not *known* to be free from any history of abortion, the results obtained could not be regarded as allowing a definite opinion to be formed as to the degree of non-specific agglutinins present in the sera of normal cattle, but they provided a reasonable basis for the practical application of the test.

These workers themselves took the view that the steer whose serum showed complete agglutination at 1 in 200, and later at 1 in 500, had actually at some time been infected with B. abortus.

The experimental results allowed them to consider that water-clear agglutination at 1 in 50 was strong evidence of infection.

In 1919 Seddon[5] reiterated the importance attaching to the determination of the greatest concentration of non-specific agglutinins present in the sera of non-infected cattle, and, in default of the examination of the sera of a large number of healthy animals, drew attention to the break in the table of agglutination reactions of a herd which he had tested. Five gave a titre of 1 in 20, none of 1 in 50, and then two of 1 in 100. At no other part of the table was there so marked a break, and he considered it reasonable to assume that the reaction at 1 in 20 was due to the presence of non-specific agglutinins, and that at 1 in 100 to specific and non-specific ones. As a result of these observations Seddon at that time adopted a minimum infective titre of 1 in 100.

In recent years a much lower titre has found favour with many workers.

In 1927 Craig[6] stated that he regarded agglutination at 1 in 50 or higher as a positive reaction, and complete agglutination at 1 in 25 as suspicious when the same serum gave an incomplete reaction at 1 in 50.

Having had some experience in the application of the agglutination test, the writer was anxious to investigate further the part played by non-specific agglutinins, but, as bovine contagious abortion is so widespread throughout the British Isles, he was under the impression that it would not be possible to find abortion-free herds whose sera could be examined.

However, as a result of preliminary inquiries from Mr. T. Le Q. Blampied, F.R.C.V.S., of Jersey, he found that the incidence of contagious abortion was very slight in that island. Later, on personal inquiry, in conjunction with Mr. Blampied, it was ascertained that there were numerous pedigree herds which had never had any cases of contagious abortion, and which had never, so far as their owners could ascertain, been in contact with any infected animals.

Cattle breeding is carried on under such rigid precautions in Jersey that each herd may be regarded as a separate unit. The absence of open markets, the tethering of cattle out at pasture, and the careful investigation of the history of any animal before its ownership is changed, all conduce to the maintenance of infection-free herds.

The individual attention which the animals receive allows us to place full confidence in the careful records kept by the owners who have kindly placed their herds at our disposal for the purposes of this investigation.

The sera from seven infection-free herds, comprising 334 head of cattle, were subjected to agglutination tests.

As a preliminary to the investigations, 18 strains of B. abortus, which had been subcultured at monthly intervals for varying periods, were submitted to agglutination tests, a known positive serum being used, in order to select a strain of high agglutinability against which the herd-sera could be tested.

The technique employed in the agglutination tests was the same as that described by the writer in the previous communication[7], but it must be briefly noted again here.

Each strain used was grown aerobically on agar at a *p*H between 7·6 and 7·8. Fresh subcultures were used, and the suspensions were made in ·75% saline solution, to which was added ·5% carbolic acid. The density of the suspensions corresponded with that of Tube 2 of Brown's Scale of Standards Opacity Tubes, and gave a measurement of 2.8 cm. by Gates' Method of Standardisation. Dreyer's agglutination tubes were used, and the volume of serum-antigen in each tube was maintained at one cubic centimetre. The usual sterile calibrated pipettes were used, and all tubes set up were incubated at 37° C. for 24 hours. The reactions were controlled by negative sera.

Explanation of signs used:

+ = Water-clear supernatant fluid, and deposit.
AC = Almost clear supernatant fluid, and deposit.
S = Slight clearing and slight deposit.
VS = Very slight clearing and very slight deposit.
T = Trace of agglutination, judged by deposit only.

AGGLUTINATION TESTS.

Strain.	*Isolated.*	1/25	1/50	1/100	1/200	1/400	1/500	1/1000	1/2500	1/5000
No. 1	Dec. 1908	+	+	+	+	VS	—	—	—	—
No. 2	Jan. 1912	+	+	+	+	AC	S	—	—	—
No. 3	Oct. 1915	+	+	+	AC	S	S	—	—	—
No. 4	Nov. 1917	+	+	+	+	S	S	—	—	—
No. 5	Jan. 1920	AC	S	—	—	—	—	—	—	—
No. 6	Oct. 1920	+	+	+	+	VS	VS	—	—	—
No. 7	Apr. 1923	+	+	+	+	+	VS	—	—	—
No. 8	Mar. 1924	+	+	+	+	+	S	VS	—	—
No. 9	Oct. 1924	+	+	+	+	AC	S	—	—	—
No. 10	Feb. 1925	+	+	+	AC	VS	—	—	—	—
No. 11	Jan. 1926	+	+	+	+	+	+	—	—	—
No. 12	Apr. 1926	+	+	+	+	+	AC	—	—	—
No. 13	Apr. 1926	+	+	+	+	AC	S	—	—	—
No. 14	Jun. 1926	+	+	+	+	AC	VS	—	—	—
No. 15	Aug. 1926	+	+	+	AC	S	S	—	—	—
No. 16	Oct. 1926	+	+	+	+	—	—	—	—	—
No. 17	Feb. 1927	+	+	+	+	S	—	—	—	—
No. 18	July. 1927	+	+	+	+	+	+	VS	—	—

All strains were tested against the same positive serum.

It will be noted that agglutinability is by no means proportionate to the length of the period during which the organisms were sub-cultured, although the most recently isolated strain (No. 18) showed the quality most strongly. A repeat test with a serum of different origin gave parallel results, and strain 18 was therefore selected for the preparation of the agglutination antigen against which the sera of the seven herds were tested.

Herd Tests.

The antigen was prepared in the manner already described, and the agglutination tests were controlled by a known positive serum.

As negative sera were being dealt with, only the lower dilutions were put up for test, the range being as follows :—

1 in 10, 15, 20, 25, 30, 50 and 100.

As no useful purpose would be served by recording in detail all the reactions obtained, only the sera (from each herd) showing maximum and minimum reactions are tabulated, together with a note of the number of sera which gave no reactions at all.

HERD A. 30 Cattle (13 cows, 16 heifers and calves, 1 bull).

No. of sera.	1/10	1/15	1/20	1/25	1/30	1/50	1/100	
1	S	VS	VS	VS	—	—	—	Maximum reaction.
5	T	—	—	—	—	—	—	Minimum reaction.

4 sera gave no reactions.

HERD B. 26 Cattle (15 cows, 10 heifers and calves, 1 bull).

No. of sera.	1/10	1/15	1/20	1/25	1/30	1/50	1/100	
1	S	VS	VS	VS	—	—	—	Maximum reaction.
2	T	—	—	—	—	—	—	Minimum reaction.

9 sera gave no reactions.

HERD C. 65 Cattle (24 cows, 38 heifers and calves, 3 bulls).

No. of sera.	1/10	1/15	1/20	1/25	1/30	1/50	1/100	
2	AC	S	VS	VS	VS	—	—	Maximum reaction.
7	T	—	—	—	—	—	—	Minimum reaction.

5 sera gave no reactions.

HERD D. 25 Cattle (14 cows, 11 heifers and calves).

No. of sera.	1/10	1/15	1/20	1/25	1/30	1/50	1/100	
1	+	AC	S	VS	T	—	—	Maximum reaction.
4	T	—	—	—	—	—	—	Minimum reaction.

8 sera gave no reactions.

HERD E. 70 Cattle (26 cows, 41 heifers and calves, 3 bulls).

No. of sera.	1/10	1/15	1/20	1/25	1/30	1/50	1/100	
1	AC	S	S	VS	T	T	—	Maximum reaction.
8	T	—	—	—	—	—	—	Minimum reaction.

13 sera gave no reactions.

HERD F. 47 Cattle (22 cows, 24 heifers and calves)

No. of sera.	1/10	1/15	1/20	1/25	1/30	1/50	1/100	
2	S	VS	VS	VS	T	T	—	Maximum reaction.
5	T	—	—	—	—	—	—	Minimum reaction.

4 sera gave no reactions.

HERD G. 72 Cattle (26 cows, 45 heifers and calves, 1 bull).

No. of sera.	1/10	1/15	1/20	1/25	1/30	1/50	1/100	
1	AC	S	VS	VS	VS	T	—	Maximum reaction.
3	T	—	—	—	—	—	—	Minimum reaction.

4 sera gave no reactions.

Only one serum out of the 334 tested showed a water-clear agglutination at a dilution of 1 in 10, and only five sera showed " almost clear " reactions at the same dilution. None of the sera showed even a "trace" of agglutination above 1 in 50 dilution.

From these results it may be accepted that the non-specific agglutinin content of normal bovine sera is a low one, so low in fact, that water-clear agglutination at 1 in 15 is probably indicative of the presence of specific agglutinins.

In all serological tests the arbitrary fixation of a border line, or belt between positive and negative reactions is inevitable, and the

writer suggests that if water-clear reactions at 1 in 15 are regarded as being within that belt, like reactions at 1 in 20 should be considered as definitely due to the presence of specific agglutinins, as well as possibly to non-specific ones in a minor degree.

For a variable period during the incubation of the disease, and also for a period during its decline, the specific agglutinins formed in the serum of an animal may be masked by the presence of the non-specific ones, and therefore a water-clear reaction at a dilution of say 1 in 10, may in some cases be due to the presence of the former, and in others to the presence of both, or to the latter only.

This information upon non-specific agglutinin content does not, therefore, allow one to infer that specific agglutinins only manifest themselves at 1 in 20 and higher dilutions, but the possible presence of non-specific ones does not allow of definite diagnostic use being made of water-clear reactions given by titres below this level. Nevertheless, when such reactions are obtained, it is advisable to re-test after a lapse of fourteeen days. A rise in titre after this period of time would be definite evidence of infection, and therefore re-testing in such cases is of the utmost importance.

The writer suggests that in a routine use of the agglutination test, dilutions of 1 in 15, 1 in 20, 1 in 40, and 1 in 60 should be put up. A serum which gives a water-clear agglutination at 1 in 20 should give complete or partial agglutination at higher dilutions, and therefore, if any serum gives a complete reaction at 1 in 20 and negligible reactions at higher dilutions a further sample or samples should be tested before any decision is arrived at. A serum which gives a water-clear reaction at 1 in 20, and an " almost clear " at 1 in 40 dilution, should, in the opinion of the writer, be regarded as positive, even if the reaction at 1 in 60 dilution is of a very slight nature. A serum which gives a water clear reaction at 1 in 15 and incomplete reactions at higher dilutions should be regarded as doubtful, and as already advised, further tests should be made.

The existence of " inhibition zones " is of importance. Freshly drawn sera sometimes fail to show agglutination in concentration and in low dilutions, although marked agglutination may occur in higher dilutions. It is unnecessary here to deal with the explanations of this phenomenon which have been advanced by Ehrlich and other workers who belong to the same school of thought, but the possibility of such zones leading to the erroneous interpretation of agglutination reactions must be guarded against. In cases where the degree of agglutination does not run parallel to the dilution gradations, or where a series of " almost clear " reactions are shewn, higher dilutions of serum should be made in order to determine whether or no an " inhibition zone " is present.

The presence of specific agglutinins can only be regarded as indicative of infection in the case of animals which have not been vaccinated with either live or dead abortion bacilli, as both cause agglutinin formation, though of a transient nature in the case of the latter.

The intradermal injection of a dose of 0·2 c.c. of a thick suspension

of dead bacilli, as used in an intradermal test for the diagnosis of the disease[7], causes the production of specific agglutinins, and a water-clear reaction as high as 1 in 25 dilution may be shewn 14 days later, which may persist for another 14 days and then rapidly decline.

That infected animals may respond to an intradermal injection at a time when their sera does not give diagnostic agglutination has been shewn in a previous communication[7], indicating that infection may be present in an animal whose serum has an extremely low specific agglutinin content.

It is necessary to point out that a strain of B. abortus of high agglutinability should be used in the preparation of agglutination antigen and that the latter must be standardised in exactly the manner stated here if accurate use is to be made of the information which has been obtained.

It is hoped that these observations will lead to an increase in the reliability of the results obtained by the use of the agglutination test.

In conclusion the writer wishes to express his great indebtedness to Mr. T. Le Q. Blampied, for making arrangements with the various owners and for taking the blood samples with such care. Without his willing help the work could not have been accomplished.

He also wishes to thank Professor J. M. Beattie, for providing him with laboratory facilities, and for his interest in the investigation.

Thanks are also due to the Director of Veterinary Research, Ministry of Agriculture and Fisheries, for supplying various strains of B. abortus, and last but not least, to the owners who have permitted blood samples to be taken from their herds.

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CANCER IN THE UDDERS OF COWS.

By J. Drabble, B.V.Sc.,
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In a previous article[1] (see this Journal, Vol. XXXIX, 1926, pp. 247-250) it was suggested that, although cancer in the udder of the cow is generally considered to be of extremely rare occurrence, a more extensive use of the microscope in the diagnosis of udder lesions in the cow might lead to a modification of this view.

At the time that the article was written one hope that time might be found to go more fully into the histology of udder lesions in cows passing through the State Abattoir ; but owing to pressure of other work it has not been found possible up to the present time to give more than the usual routine attention to udder lesions.

In spite of this, however, three additional cases of cancer in the udders of cows have been met with.

Two of these were marked by the writer as " suspect " for cancer on ante-mortem inspection at the Flemington saleyards, and were followed up on the slaughterhouse floor and in the laboratory. The third case was marked as " suspect " on ante-mortem inspection for cancer of the vulva and anus ; but, as this was a beef animal, the lesions in the udder and supramammary lymphatic gland were not discovered until the post-mortem examination was made.

It might be mentioned here that our beef cattle are wild, and cannot be handled or safely approached unless placed in a crush.

The particulars of the three cases are as follows.

Case I.

This was a red and white dairy cow, about six years old and in fairly good condition. The udder was affected with chronic mastitis ; and in the left posterior quarter was an irregular breach or open wound, about 5 c.m. in diameter, which emitted a very repulsive odour, looked very sore, and from which a thin greyish-brown discharge slowly dripped. Numerous fly larvæ were present in the open lesion. On palpation the left supramammary lymphatic gland was found to be slightly enlarged.

Post-mortem examination showed the udder to be in a very repulsive condition. The left posterior milk sinus was filled with loose débris and fly larvæ. The surface of the sinus was eroded and nodular ; and the surrounding normal udder tissue had been replaced by a very firm, cream-coloured tissue which extended into the gland from the sinus for distances which on the section surface varied from one to 5 c.m. The left supramammary lymphatic gland was enlarged and invaded by firm cream-coloured tissue similar to that noticed invading the udder.

Microscopic examination of paraffin sections made from the lesions in the udder showed the growth to be a rapidly growing cancer. Mitotic figures were very numerous. In some cases the nuclei had divided a number of times without separating, thus giving rise to small mulberry-like masses of nuclei. This was especially noticeable in the section of which the microphotograph shown in Fig. 1 was taken. Here the epithelium can be seen invading the

udder from the mucosa of the milk sinus. It is probable that invasion of the udder had also taken place from the skin.

Fig. 2 shows a microphotograph of a section of a deeper portion of the uddergrowth. In this a few acini of the gland have been purposely included, and the growth can here be seen to be definitely squamous-celled cancer.

The appearance under the microscope varied considerably in different parts of the growth. In some sections the tendency to " pearl " formation

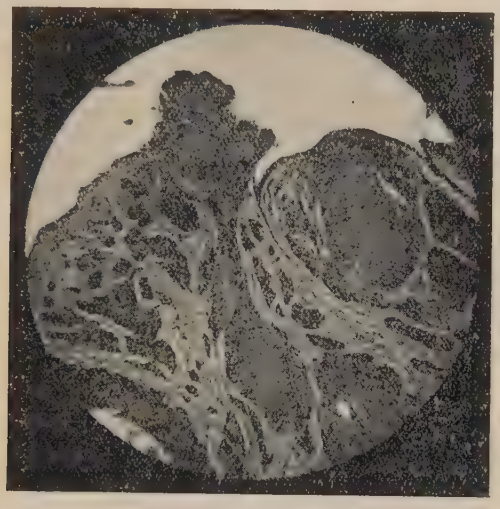

Fig. 1.—Case 1.

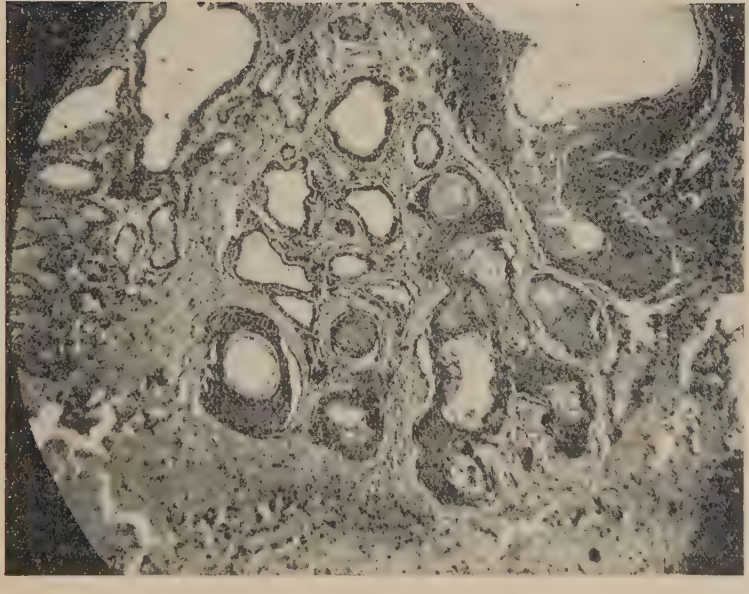

Fig. 2.—Case 1.

was almost or entirely absent, and the epitheliomatous nature of the growth was not very obvious. In others, however, the cell masses contained many prickle cells, and " cell nests " or " pearls " were numerous and well formed.

Examination of sections of the lesion in the affected lymphatic gland also exhibited evidence of the activity of the growth. Mitotic figures were very numerous. Mononuclear giant cells had been formed, and also other large cells, which, while resembling mononuclear giant cells, were really multinuclear, but the nuclei had not separated after dividing.

A short distance from the growing epithelium, in some fields, both in the udder and in the lymphatic gland, large irregular foreign body giant cells were noticed.

Case II.

Dairy cow, white body, roan head and neck ; broken mouth ; fair condition ; marked as " suspect " for cancer of the udder. The upper part of the posterior surface of the udder showed a more or less oval area which was covered by a thin, dry brown crust. This area measured approximately 5 c.m. by 3 c.m., and had a slightly concave surface. The surrounding tissue was very firm, and this abnormal tissue apparently extended into the udder for a considerable distance.

On post-mortem examination of the udder the malignancy of the lesion was fairly obvious even to the naked eye. The centre of the organ was occupied by a quantity of débris formed apparently by the breaking down of portions of the growth. Elsewhere the growth was very firm in consistence, and the surface of incision exhibited the mottled appearance so frequently seen in cases of cancer.

The right supramammary lymphatic gland was greatly enlarged, and its section surface also showed that the gland was extensively invaded by the growth.

Examination of suitably stained sections under the microscope showed that the growth in the udder and supramammary lymphatic gland was typical squamous-celled cancer. The tendency to " pearl " formation in the udder was very marked, and in the affected lymphatic gland was extreme. In some fields the " pearls " consisted entirely of squamous cells, the outer basal cells having either disappeared or become squamous. Some of the pearls were very large and many were confluent. A few small areas of calcification were noticed in the affected lymph gland, and the microscopic appearances suggested that the growth in the lymph gland was somewhat older than the growth in the deeper parts of the udder. It is probable that the lymphatic gland was invaded directly and very early by the epithelium growing inwards from the skin lesion on the posterior surface of the udder, as this lesion, which the writer regards as the primary one, was situated approximately over the spot where one palpates the supramammary lymphatic glands when making an ante-mortem inspection.

In Fig. 3 the epithelium is shown growing inwards from this surface lesion.

Case III.

White beef-shorthorn cow ; three years old ; good condition ; marked as " suspect " for cancer of the anus and vulva. The vulva was affected on both sides, and the anus showed on the right side a rounded projecting growth about 4 c.m. in diameter.

FIG. 3.—Case 2.

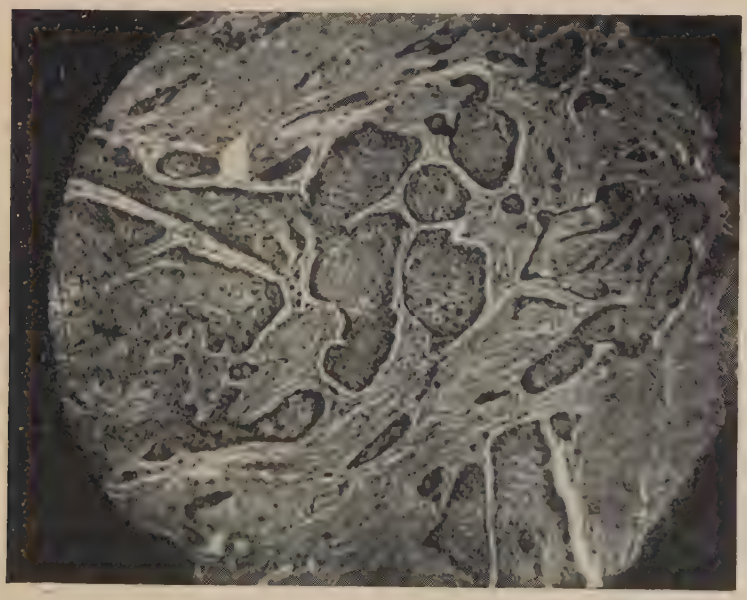

FIG. 4.—Case 3.

Post-mortem examination showed that the growth in the wall of the vulva was well established, and had extended in an upward direction to the right side of the anus, but was not continuous with the anal growth previously mentioned. Both ischiatic lymph glands were enlarged, and showed naked eye lesions. The udder was very small, even for a young beef animal, and was firmer than

normal. When incised it was found that both the udder and the right supramammary lymph gland were invaded by a growth which was somewhat more extensive in the lymph gland than in the udder. The lesion in the lymph gland was directly continuous with that in the udder.

Sections of the anal growth showed it to be typical squamous-celled cancer, with well formed " pearls." Just in advance of the growing points of some of the columns of epithelium descending from the surface, in most sections, were some beautifully formed multinuclear giant cells, with nuclei arranged in the horse-shoe formation so often seen in tuberculous lesions. Out of curiosity sections were stained for acid-fast bacilli, but the results were negative.

Sections of the growth in the wall of the vulva showed it to be typical squamous-celled cancer.

Microscopic examination also showed that the growth in the udder and supramammary lymph gland was squamous-celled cancer. A microphotograph of a section made from the udder growth is shown in Fig. 4.

This case is an extremely interesting one, not only on account of the early age at which the animal was found to be affected, but also because there seems to be a strong probability that the supramammary lymph gland was invaded, not from the udder, but from the vulva, and that the udder was invaded from the supramammary lymph gland. The evidence in favour of this may be summarised as follows :—

(1) The growth in the walls of the vulva was very obviously the oldest lesion in the animal under discussion.
(2) The supramammary lymph gland receives afferent vessels from the vulva.
(3) The supramammary lymph gland contained more growth than did the udder.
(4) The growth in the udder was directly continuous with that in the supramammary lymph gland.

General Remarks.

When one remembers that epithelioma of the human female breast is very uncommon, it becomes a matter of interest that the four cases of cancer that have been met with in the udders of cows at the New South Wales State Abattoir have proved to be of the squamous-celled type. However, the writer believes that, sooner or later, other types will be met with, not only in New South Wales but also in other parts of the world.

If anyone should ultimately succeed in proving cancer to be infectious, the fact that cancer does occur in the bovine udder, and the probability of its incidence in this situation being greater than was formerly supposed, would immediately cause far more expert attention to be given to the inspection and supervision of dairy cattle, and of all matters connected with the dairying industry, than is given in most countries at the present time.

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A CASE OF EXTRA-UTERINE GESTATION IN A SOW.

By J. Drabble, B.V.Sc.,
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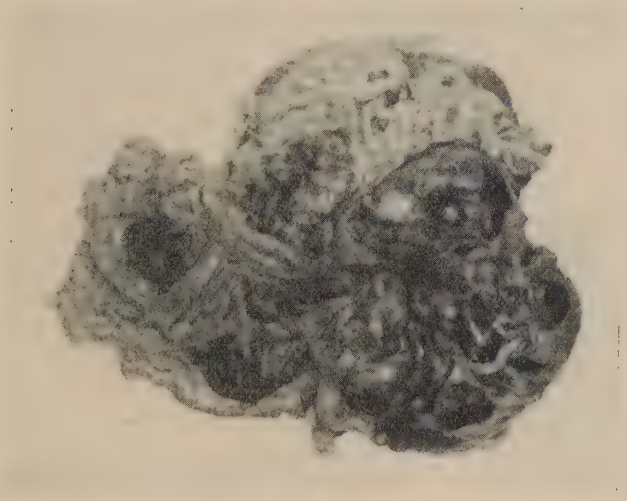
Cases of extra-uterine gestation are apparently not very common in food animals, as, in spite of the very large numbers of cattle, sheep, and pigs killed annually, the only case met with up to the present time at the New South Wales State Abattoir is the one herein described.

One day when the writer was passing through the pig slaughtering units at the State Abattoir he noticed an Inspector at one of the inspection tables intently studying something that was apparently unusual.

On investigation it was found that the organs had just been removed from a Berkshire sow, and, wrapped snugly in the omentum, was a dark trilobed mass about 17·5 c.m. (7 inches) in diameter. The lace-like omentum was firmly adherent to the mass, which, on closer examination was seen to be formed of three fœtuses. The fœtal pigs were of equal size, firmly adherent to each other, and were advanced in development, even the hair being present. Evidently they had been in the abdominal cavity for a considerable time, and were beginning to show signs of mummification. Each was more or less curved upon itself dorso-ventrally.



A straight line from the point of the ischium to the forehead of each fœtus measured 13·75 c.m. (5½ inches). The distance between the same points measured along the curve of the body was 17·5 c.m. (7 inches).

All of the sexual organs of the mother were apparently quite normal. The uterus was in the non-gravid condition.

In this case it is a matter for speculation as to whether impregnation took place actually in the abdominal cavity, or in the ovarian end of the

Fallopian tube. From the latter position the fertilised ova could have been jolted into the abdominal cavity by a sudden fall of the mother.

The photograph shows the three fœtal pigs in the omentum, a portion of which has been dissected away in order to give a clear view of one fœtus.

A NOTE ON TUBERCULOSIS IN THE FERRET.

By G. W. Dunkin, P. P. Laidlaw, and A. S. Griffith,

From National Institute for Medical Research Farm Laboratories, and Cambridge University Field Laboratories.

During the course of an investigation into Dog Distemper, two of us (G.W.D. and P.P.L.) have encountered three cases of spontaneous tuberculosis in the ferret. The findings in these cases, together with the results of a few experimental investigations, appear to us to be worthy of record.

Case of Spontaneous Tuberculosis.

In all the spontaneously infected ferrets the mesenteric glands were greatly enlarged, matted together, and on section showed fibrosis with patches of caseation definitely softened in places. The mesenteric gland lesions in each case were clearly the oldest in the body and strongly suggested that infection had occurred from the alimentary canal.

The first ferret, which was killed with chloroform when exceedingly ill, showed in addition to the mesenteric gland lesions, very extensive tuberculosis of the lungs, which were in a condition of caseous pneumonia. The liver and spleen, which were not examined microscopically, appeared to be healthy.

The second ferret, which died from the disease, showed in the lungs only a few tubercles, apparently of quite recent formation. On the other hand, there was widespread disease of the liver, which contained tubercles of all sizes scattered throughout its substance; in places groups of larger tubercles were to be seen packed closely together, forming aggregations 6 or 7 mm. in diameter. There were also numerous tubercles of various sizes in the spleen, and numerous small tubercles in the peritoneum over the kidneys, and in the omentum. At the anterior end of the thorax, on the right side, there was a caseous mass.

The third ferret, which was killed with chloroform when observed to be seriously ill, showed the lungs, liver, and spleen closely beset with tubercles of quite recent formation.

The microscopical appearances of the tissues in sections were those of acute tuberculosis. "Giant cells" were rare and of small size. Sections stained by the Ziehl-Neelsen method showed enormous numbers of acid-fast organisms in all three cases, but more particularly in the second. The bacilli were intracellular and more numerous

towards the periphery of the lesions. In sections of the liver, columns of endothelioid cells, the majority of which contained several bacilli, could be traced from the edge of the lesion in between the surviving liver cells for a considerable distance. The extent of the disease was more widespread and the organisms were more numerous than the naked eye examination of the tissues had led one to expect. In some sections the bacteria were so numerous that the appearances were only comparable with those encountered in leprosy or Johne's disease.

A guinea-pig and two rats were inoculated subcutaneously with material from the second case. The guinea-pig developed a local lesion and large lymph glands and went rapidly downhill. It was killed on the 14th day of the experiment, and found to have generalised tuberculosis. The rats gained weight and were killed three months after inoculation. At the autopsies the local lymphatic glands appeared normal to the naked eye, but were found when examined microscopically to contain a few acid-fast organisms.

Cultures of tubercle bacilli were secured from the second ferret, directly and through the guinea-pig. The cultures were dysgonic, and the direct strain in a dose of 5·0 mg. inoculated subcutaneously caused the death of a rabbit in 56 days from severe progressive generalised tuberculosis. The ferret was thus proved to have been infected with tubercle of the bovine type.

It is considered probable that the infection in these three ferrets was due to feeding on raw cows' milk. In one case the animal was home bred and had been fed exclusively on raw horse meat and cows' milk. In the others infection must have occurred before the animals were received, but the known custom of feeding ferrets on raw milk is consistent with the view that infection in these cases was also due to milk feeding.

Experimental Investigations.

It became of interest to determine whether ferrets are susceptible to the human as well as the bovine type of organism, and for that purpose a culture of each type was employed. The bovine strain (No. 17 B.V.) had been under artificial cultivation nearly three years, and was rather less virulent for the rabbit than is usual for bovine strains. The human strain had been recently isolated from the cerebro-spinal fluid of a woman who died of acute tuberculous encephalitis and it was of low virulence for the rabbit. They may be regarded as typical representative strains, although not the most divergent it is possible to secure.

Short summaries of the experiments are given below :—

F. 291/27 was injected subcutaneously with 1·0 mg. of bovine tubercle bacilli. There was rapid development of a local lesion which in fourteen days was a mass one inch in diameter. Death ensued at the end of the seventh week. Post-mortem findings showed generalised tuberculosis, with enormous numbers of bacilli in all the lesions.

F. 292/27 was given 1·0 mg. of bovine tubercle bacilli by the mouth and was killed with chloroform after 18 weeks. The autopsy showed matted fibro-caseous glands at the root of the mesentery and early generalised tuberculosis

Numerous acid-fast organisms were found in the glands, lungs, and spleen, and a smaller number in the liver.

F. 293/27 was injected subcutaneously with 1·0 mg. of human tubercle bacilli. No local mass was palpable fourteen days later when the animal succumbed to an empyema. Post-mortem examination revealed a small red local lesion and congestion with slight enlargement of the neighbouring lymphatic glands, in which a few acid-fast organisms were found. No tubercle bacilli were found in the thorax or elsewhere in the body.

F. 294/27 was given 10·0 mg. of human tubercle bacilli by the mouth and was killed with chloroform after eighteen weeks. The autopsy showed enlargement of the mesenteric glands and no sign of tuberculosis in the lungs, liver, spleen, or kidneys. Microscopical examination showed a few acid-fast organisms in sections of the intestinal lymphatic glands, but none could be found elsewhere.

F. 24/28 was injected subcutaneously with 5·0 mg. of human tubercle bacilli. A small local swelling developed in five days, which softened, broke, and left an indolent ulcer. This ulcer slowly increased in size and the adjacent glands underwent moderate enlargement. The ferret remained in good general condition and was killed two months after inoculation. The autopsy showed the superficial glands to be slightly enlarged. The spleen was of very large size, but showed no sign of tuberculosis. The liver, lungs, and kidneys seemed normal. Behind the sternum there was a caseous lymph gland. Tubercle bacilli were found in the local lesion and also in small numbers in the lymph glands. None could be found in the lungs, liver, or spleen.

F. 25/28 was injected subcutaneously with 1·0 mg. of human tubercle bacilli and was killed seven weeks after inoculation. The clinical course of the disease was similar to that in F. 24/28. The autopsy showed the same type of ulcer and the superficial lymphatic glands alone appeared to be tuberculous. The spleen was very large, but free from tuberculosis. The retro-sternal lymphatic glands were not infected in this case. Microscopical examination showed a few acid-fast organisms in the local lesion and in the lymph glands. The liver, spleen, and lungs appeared to be free.

If the results of these six experiments are examined, it will be seen that there is a very great difference in the effects produced by the two types of bacilli. F. 291/27, infected by subcutaneous injection with bovine organisms, showed rapid development of disease and died of generalised tuberculosis in seven weeks. Tubercle bacilli were numerous in all the lesions. F. 24/28 and 25/28, on the other hand, which were injected subcutaneously with the human type of organism, were never ill, and were quite fat when killed two months and seven weeks respectively after inoculation ; they might probably have been kept alive much longer, but it was thought desirable to terminate the experiment because the indolent ulcers which formed at the injection site were continually fouled by bedding and showed no signs of healing. Macroscopic tuberculous lesions in each case were confined to the seat of inoculation and the adjacent lymph glands, and microscopically no lesions or tubercle bacilli were found in the lungs, liver, or spleen.

On comparing the results of feeding experiments one finds the contrast between the two strains again striking. Infection with the bovine type of organism was followed in seventeen weeks by general tuberculosis, with many bacteria in the lesions ; whilst with the human type, even although ten times the dose was administered,

there was no generalisation in seventeen weeks, and the only site where acid fast bacteria could be detected was the mass of mesenteric lymph glands, and here they were not numerous.

It seems clear that the ferret is highly susceptible to the bovine type, and relatively insusceptible to the human type of tubercle bacillus.

Summary.

(1) Spontaneous tuberculosis in the ferret is recorded.

(2) The type of infecting bacillus was determined in one instance and found to be bovine.

(3) Bovine tubercle bacilli are more virulent for the ferret than human tubercle bacilli.

SOME EXPERIMENTS ON TUBERCULIN TESTING BY THE SUBCUTANEOUS AND INTRADERMAL METHODS, AND OBSERVATIONS AS TO THEIR COMPARATIVE VALUES ON YOUNG CATTLE AND DAIRY COWS, RESPECTIVELY.

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During the year 1922, while examining a number of carcases and internal organs of dairy cows, which, immediately prior to slaughter, had been subjected to the subcutaneous tuberculin test, the writer was surprised to find lesions of tuberculosis in a considerable proportion of those which had given no reaction whatever. On giving the matter consideration, it was decided to submit a number of apparently healthy dairy cows and other cattle, including those which had been repeatedly tested, to the subcutaneous test and compare the results with those of the post-mortem examinations conducted immediately afterwards, with the view of making a search for the presence of tuberculous lesions discernible in any degree. The animals used in these experiments, with the exception of one heifer belonging to a wholesale meat trader, comprised part of a herd of cattle, mostly shorthorns, kept at the Philipson Farm Colony, Stannington, Morpeth, Northumberland, for the purpose of supplying milk for the patients (tuberculous children) housed within the Stannington Sanatorium. The entire herd had been tested from time to time for the purpose of isolating reactors from non-reactors. Irrespective of the testing, the animals, particularly the cows in milk, were subjected to regular inspection for the purposes of the milk supply, and, although samples of the latter have been submitted from time to time to guinea-pig inoculation, in no instance has any been found to give a positive result.

The work on the subcutaneous method of testing was commenced in the beginning of 1923, and continued unti lthe appearance of a Report on comparative tests and also on the combined ophthalmic and intradermal methods by the Tuberculin Committee appointed by the Medical Research Council in 1925. It was then decided to include experiments on the intradermal method and, if possible, to carry out an equal number of each. Up to November, 1927, 86 experiments had been completed, 50 by the subcutaneous method and 36 by the intradermal method. As it has not been found convenient to carry out the total number originally intended, a record of the work completed is submitted, together with some observations on experiments by other workers, to form the subject of this article.

It is perhaps true to state that the clearer one understands the shortcomings of the various methods of tuberculin testing, as applied to cattle of all ages and under varying circumstances, the better will one appreciate the true value of tuberculin as a diagnostic agent. For many years a common complaint against the use of tuberculin has been that sometimes when an animal, subcutaneously injected, had definitely reacted and was slaughtered soon afterwards it was found to be free from disease. It is certain that in the great majority of such cases the tuberculin was not at fault, and that any wrong diagnosis arrived at was due either to an imperfectly conducted post-mortem examination or failure to recognise that, although the animal was diseased, the lesions had not yet reached macroscopic dimensions. According to Melvin, out of 23,869 reacting animals slaughtered, lesions of tuberculosis were found in 23,585, or 98·81 per cent., whilst Arloing affirms that in all cases where a tissue reaction is obtained we can assert that there is a tuberculous infection of the animal. Heymans, like many other observers, has also found that a number of reacting animals fail to show macroscopic lesions, but his experience proves that the disease is present in such cases ; to this observer failure to de ect evidence of the disease in reacting animals after slaughter is indicative of the imperfectness of the macroscopical examination. Again, according to Calmette, every definite reaction which is 1° C. or more above the maximum normal should lead to the conclusion that a tuberculous focus exists ; but the latter may be so very small that, on killing and autopsying the animal, it is at times found only with the greatest difficulty It was proven, however, long ago, that in these circumstances the organs had not been searched with sufficient care and that, whenever a tuberculin reaction is positive, there exists somewhere a follicular lesion or at least a gland containing bacilli whose presence can be disclosed by experimental inoculation into the guinea-pig.

During the past five years the writer has examined, post-mortem, 86 cows which were known to have given a positive reaction to the subcutaneous test and in 85, or 98·83 per cent., lesions of tuberculosis were found. In the one case in which lesions were not detected the animal had reacted, then ceased to react seven months later, and was slaughtered the following year, but neither microscopical nor biological examinations were made.

The Importance of Doubtful Reactions.

Apart from a well defined or positive reaction, reactions are frequently recorded as doubtful. For example, the pre-inoculation temperature may register, say, 101·4° F. and continue at that from the time of injecting the tuberculin until perhaps the 12th, 15th, or even 18th hour afterwards, when it is found to register 103·4° F. or therabouts and revert to the normal as at first registered. The experience of the author is that in such cases the animals on slaughter will invariably be found tuberculous. In Rutherford's experimental tests, it was found that of 32 cows 9 gave doubtful reactions, and in eight of these lesions of tuberculosis were found at the autopsy, whilst in 32 young animals (other than cows) seven gave doubtful reactions, in three of which tuberculous lesions were found on slaughter. When a so-called doubtful reaction is recorded by the subcutaneous test we have highly important warning evidence as to the probable presence of disease, whether the lesions are of macroscopical dimensions or otherwise; and whenever this method is being applied along with either the ophthalmic or intradermal method, and it has registered a doubtful reaction as indicated, although either or both of the latter have given a definitely negative result, it is within the range of a practical certainty that it would be erroneous to conclude that the animal in question was free from disease. In this connection, it may be noted that in the comparative tests carried out by the Tuberculin Committee of the Medical Research Council two cows (Nos. 14 and 29, Herd C., page 38 of the Report) had given so-called doubtful reactions but did not react to either the ophthalmic of the intradermal method, whilst in their Third Investigation at Moundsmere Manor four cows (Nos. 440, 456, 476 and 478, page 56 of the Report) were tested three times by the subcutaneous and intradermal methods. At the first test in December, 1923, they all gave positive reactions to the subcutaneous method and negative results to the intradermal method. In March, 1924 they were again submitted to the two tests. They were again negative to the intradermal test, but by the subcutaneous method two were positive (456 and 478) and two (440 and 476) gave the so-called doubtful reactions. At the third test, in the following July, all were again negative by the intradermal method, whilst three were on this occasion negative to the subcutaneous test. When these animals were slaughtered, although it is not claimed that the post-mortem examinations were exhaustive, lesions of tuberculosis were, nevertheless, found in two of them, including one which had on one occasion given a so-called doubtful reaction.

Do Healthy Animals React?

In dealing with the question as to the possibility of an injection of tuberculin causing an elevation of temperature in a non-tuberculous animal, McFadyean and Sheather state that there need not be much hesitation in giving a negative answer, or in denying that there is any evidence to prove that tuberculin, even when injected in much larger

doses than those employed in testing, is capable of producing any sensible disturbance of the temperature in a non-tuberculous subject. This view appears to have been confirmed by Bang and Velling by a demonstration on 134 healthy animals, by Joseph in tests on 126 animals by the intradermal method, and by the Medical Research Council (Second Investigation, Moundsmere Manor), when 100 cows were submitted to the ophthalmic, subcutaneous, and intradermal methods of testing without obtaining any reaction. Whilst on the one hand it appears certain that the application of tuberculin to a healthy subject does not provoke a reaction, it seems on the other hand reasonable to conclude that, excluding accidents, any reaction to tuberculin, although not what is commonly accepted as typical by either the ophthalmic, subcutaneous, or intradermic methods of testing, should be regarded as a signal warning as to the probability of the animal in question being infected.

According to Lignières, writing in the year 1909, in Veterinary Medicine there seem to exist two opposite tendencies, one to attribute to the subcutaneous injection of tuberculin superiority over all other methods, the other to regard one or other of the local reactions as exclusively sufficient. For example, at the meeting of the Central Society in Paris, on October 15th, 1908, Vallée expressed himself as follows :—" In the present state of our knowledge we can only regard the different methods of examination by local reaction as makeshifts which cannot displace the classic method by the subcutaneous injection of tuberculin, the value of which is beyond question."

At the meeting of the same Society, held on December 30th, 1908, Moussu said :—" Experience has shown that subcutaneous injections and cuticular injection, as well as the ophthalmic reaction, are open to very great objections ; we must therefore seek a more perfect method. Intradermic injection seems to fulfil this need, and it is very probable that it will prove even more satisfactory in the future ; but for the moment I confine myself to repeating that it seems to me a method which will eventually replace the older systems. To Lignières neither of these opinions seems acceptable. He believes that in detecting tuberculosis it is necessary, in choosing our method carefully to appraise the conditions under which one has to work, the species of animal to be tested, the conditions under which it is kept, the facilities for observation, the condition of the subject's health, the temperature, the time of year, the security against fraud, the time during which the animals are at the disposal of the observer, the experience acquired with such and such methods, etc." One point, he states, dominates everything in the diagnosis of tuberculosis by means of tuberculin, that is, the search for a clearly defined positive reaction. Negative reactions have only a relative significance. With this statement the author more or less agrees, and, in his experience of testing dairy herds which produce reactors, the animals which provide the most interesting problem for solution are those which have always given negative results by all three methods of testing, singly or combined, and at the same time are in an apparently healthy and

thriving condition but are found to be tuberculous when killed. In considering the definitely opposed views of not only Vallée and Moussu but of many others concerning the comparative values of the subcutaneous and intradermal methods of testing, one is inclined to ask what are the conditions giving rise to such diverse opinions. For example, were the animals tested in the one case quite young and in the other from fully adult up to old age, such as dairy cows ; or were they all adults ? It seems possible, to the writer at least, that the two perfectly opposed views may be quite accurate so far as the results of the tests were actually concerned, but because no descriptions of the animals tested were given an erroneous impression may have been created. If it be found that the different methods have different values, as to reliability, when applied to quite young cattle as compared with adults, then the choice may or may not be greater when one intends to test young cattle for the purpose of eliminating the diseased animals in the building up of a tuberculosis free herd as compared, for example, with the testing of a herd of dairy cattle into which newly purchased animals are being introduced from time to time. In this connection it may be asked : Is the subcutaneous method of testing reliable when applied to calves and other young cattle ; and is the intradermal method equally or more so when applied to animals coming within precisely the same category ? Again, can any distinction be drawn between the percentage accuracy of results obtainable when either method is applied to the class of animals referred to, and that of results obtainable when applied to fully adult cattle, and particularly those that may, through housing, age, etc,. have been exposed to the risks of infection from time to time ? Finally, how do these methods of testing compare in the latter respect ?

Some Comparisons.

As far back as the years 1905 to 1909, Rutherford carried out, in connection with open-air experiments with tuberculous cattle, a number of tests by the subcutaneous method which were checked by post-mortem examinations. Seventy-one animals were tested and for convenience these may be referred to in two lots. The first comprised 39 animals, 13 being from five months to one year ; 14 between one and two years ; 10 over two and under three years, and 2 three years of age. In 28 (15 positive and 13 negative), or 71·79 per cent. of the cases, the result of the test was in agreement with the post-mortem findings. Of the tests immediately prior to slaughter, four were positive and three were negative among the animals in which no lesions were discovered, but in one which had given a doubtful reaction and three that gave negative results tuberculous lesions were found. It should be noted, however, that of the 19 animals that were found tuberculous all except two had at some time given a positive reaction. One of the two had given doubtful and the other negative results. To the last mentioned animal Rutherford directs special attention. On being slaughtered when over two years old it was found badly affected with tuberculosis, although it had never shown any

evidence of disease and had been tested four times without reaction, the periods elapsing between the tests being 10, 6, and 3 months, respectively. This case, he states, is interesting as showing that an animal, while still retaining externally the appearance of health, may, within a comparatively short period become affected to such an extent as to nullify the action of tuberculin.

The second lot comprised 32 animals, chiefly cows varying from 4 to 13 years of age. In 19 (16 positive and 3 negative), or 59·3 per cent. of the cases, the results of the test were in agreement with the post-mortem findings. Of the tests immediately prior to slaughter, two were positive, in which no lesions were discovered ; five were doubtful and six were negative, and in all of these lesions of tuberculosis were found. Of the six animals which gave negative results, one was a clinical case whilst the remaining five were what are known as " ceased reactors." It should be noted regarding this lot that a total of 27 animals were found tuberculous on slaughter, and that in every case the animal at some period had given a positive reaction.

Therefore, in 46 cases (old and young animals) where post-mortem lesions were found, 44 had given definite positive reactions, one a doubtful reaction, and one animal, to which special attention has been directed, gave a definitely negative result. As Rutherford states, the most striking feature of the whole of the tests is the fact that 16 mature animals (cows) which positively reacted in from one to four tests became " ceased reactors " but subsequently reacted again.

It will be observed regarding these tests that the percentage of cases in which the tests were in agreement with the post-mortem findings was much higher in the young animals than that concerning the older animals ; besides, in the tests on the young animals only three were negative immediately prior to slaughter in animals found tuberculous, compared with twice that number in the case of the older animals, although the total number of the latter tested was considerably less than that of the former. Therefore, comparing the results of the tests carried out immediately before slaughter, it would appear that the test is more reliable when applied to quite young animals than when applied to adults, such as cows.

Nearly twenty years ago, Joseph applied the intradermal test to 126 animals (in five series) with remarkably successful results. Of the whole, 78 gave positive reactions, and in 77 of these and in one in which the reaction was considered doubtful lesions of tuberculosis were found when the animals were slaughtered. In one animal which had given a positive reaction no lesions were found, and none were found in any one of the 48 animals which did not react. As the ages and description of the animals are not given, it is not possible to compare these results in that respect with those under discussion. Notwi hstanding this, however, the results obtained appear to stand out prominently, particularly as not a single non-reactor was found after slaughter to be diseased, as the most successful by this method of testing yet recorded.

Ward and Baker carried out experiments with the intradermal test on 100 range cows, slaughtered immediately afterwards. In 28 cases in which the test was positive and in three in which it was doubtful tuberculous lesions were found at the post-mortem. In 59 negative, 2 positive, and one in which the test was doubtful, no lesions were found. In seven cases, however, in which the results of the test were all negative tuberculous lesions were found when the animals were slaughtered.

McFadyean and Sheather carried out a large number of experiments regarding different methods of tuberculin testing on young cattle which they had previously infected with tubercle bacilli. Some of these animals were calves six months old, whilst several had reached the age of over two years before the final test. In addition to these, four cows, which were naturally infected animals and were all found tuberculous on slaughter, were included in their experiments. These observations, have reference, so far as the young cattle are concerned, only to the animals infected with bovine bacilli and dealt with as seven series. In six of the seven series (mostly calves), comprising a total of 50 animals (series 1, 2, 3, 4, 5, and 7) submitted for the first time to the subcutaneous, conjunctival, and intracutaneous methods of testing, applied simultaneously and human tuberculin being used, the following results were obtained : —

(i) By the subcutaneous method, all reacted. It should be added, however, that in series IV, 2 of the animals had high initial temperatures. Notwithstanding this, these animals appear to have reacted also.

(ii) By the conjunctival method, 16 gave negative results ; one appears to have been doubtful whilst two animals were not tested.

(iii) By the intracutaneous method, all reacted except one in series I which was entirely negative.

Therefore, in comparing the different methods, although used in combination, as to their respective values, in the event of one testing a herd of young cattle for the first time by any one of the methods with the view of detecting those affected, it would appear, on the assumption that all the animals were diseased (as was the case with these) and that the tests applied alone would have given precisely similar results, none could have escaped by the subcutaneous method, one by the intracutaneous method, and 16 by the conjunctival method. Again, the animals included in series VI were submitted to repeated tests over a period of some months with varying intervals of weeks, with the result that, taking all the methods and tests applied (including series VI amongst which were nine animals tested with bovine tuberculin), a total of 142 tests by the subcutaneous method, 140 tests by the conjunctival method, and 124 tests by the intracutaneous method were carried out.

Of the 142 subcutaneous tests, 134 were positive, five were negative (in three avian tuberculin was used), and three were doubtful (in one bovine and in two human tuberculin was used).

Of the 140 conjunctival tests, 86 were positive, 50 were negative (in three avian tuberculin was used) and four were doubtful (in two bovine tuberculin was used).

Of the 124 intracutaneous tests, 117 were positive, six were negative, and one was doubtful.

If the three subcutaneous tests, negative with avian tuberculin, be eliminated for purposes of comparison, then it may be stated that out of 139 subcutaneous tests applied there were only two in which the reactions were negative and three in which they might be considered as doubtful, as compared with six tests in which the reactions were negative and one which might be considered as doubtful out of the 124 tests by the intracutaneous method. The conjuctival method gave results very far behind the intracutaneous method in the matter of accuracy. Now, on turning to the experiments on the four cows, one finds that as compared with those on the young cattle, the comparative results give quite a different impression. For example :—

Cow No. 1 was submitted to 24 tests, conjunctival, subcutaneous, and intracutaneous, singly or combined. There were two negative results and the method of testing which gave these was the subcutaneous.

Cow No. 2 was submitted to 14 tests, conjunctival, subcutaneous, and intracutaneous, separately or combined. Two of the results were negative and one was doubtful. The former were obtained by the subcutaneous and the latter by the intracutaneous method.

Cow No. 3 was submitted to 11 tests, conjunctival, subcutaneous, and intracutaneous methods, separately or combined. There was one negative result by the conjunctival method and one doubtful result by the intracutaneous method ; and

Cow No. 4 was tested twice by the conjunctival method, a positive reaction being obtained on each occasion.

It will be observed, therefore, that cows Nos. 1, 2, and 3 were submitted to a total of 49 tests, namely :—

Seventeen by the subcutaneous method, with a negative result on four occasions.

Twenty-two by the conjunctival method, with a negative result on one occasion ; and

Ten by the intracutaneous method, with a doubtful result on two occasions.

When one compares the subcutaneous and intracutaneous methods of testing by the results of these experiments one finds that in the tests on young cattle the subcutaneous method was proved to be the more reliable, whereas when cows were being tested the intracutaneous was proved to be the more reliable method of the two.

During the years 1922 to 1924, the Tuberculin Committee of the Medical Research Council carried out a large number of comparative tests on several hundred animals, chiefly cows, in ten investigations, besides submitting a total of 114 animals (bulls, cows, steers, heifers, and calves) to the combined ophthalmic and intradermal methods of testing.

Of the former, only about half a dozen of the animals were slaughtered, whereas the whole of the latter were slaughtered on the completion of the tests and subjected to post-mortem, including microscopical and biological examinations. Owing to the fact that the animals used in the comparative tests were not slaughtered, it would

be difficult to estimate to what extent the results by any one of the three methods employed were accurate or inaccurate. Under these conditions, it would appear that to assess the accuracy of one method by the result of another would simply mean determining the value of one method of testing by the results of another the accuracy or reliability of which was not known. For example, if one tested, say, six cows by two methods, one of which gave three clearly defined positive reactions whilst the other gave equally definite negative results, the case would be only half proven in the absence of a post-mortem examination, for the reason that one or more of the three non-reactors may have been diseased.

On comparing the subcutaneous and intradermal methods of testing by counting the negative results obtained by the method under consideration as errors against positive reactions at a previous test by either method on the same animals, we find that :

When 112 animals (Herd A, B, D, E, Third Investigation, Moundsmere Manor, and Herd G) were tested by the subcutaneous method, 26, or 23·2 per cent. errors were recorded ; and

When 98 animals (Herd C, E, F. Third Investigation, Moundsmere Manor, and Herd G) were tested by the intradermal method, 13, or 13·2 per cent, errors were recorded.

Again, in comparing these two methods by counting negative results of the test under consideration as errors against positive reactions obtained by some other method, either previously, at the same time, or at a subsequent test on the same animals, we find that :—

When 261 animals (Herd A, B, Second Investigation, Moundsmere Manor, Herd C, D, E, Third Investigation, Moundsmere Manor, and Herd G) were tested by the subcutaneous method, 38, or 14·1 per cent., errors were recorded, whereas when 319 animals (Herd B, Second Investigation, Moundsmere Manor, Herd C, D, E, F, Third Investigation, Moundsmere Manor, and Herd G) were tested by the intradermal method, 23, or 7·2 per cent. errors, were recorded.

When, however, the 114 animals (not known to have been previously tested) were subjected to the combined ophthalmic and intradermal methods of testing and submitted to pathological investigations on slaughter immediately afterwards, it was found that this method had given remarkably successful results, inasmuch as only one animal, an aged cow which had given a negative result to the test, was found tuberculous by guinea-pig inoculations.

In all the tests, the number of cows submitted to post-mortem examinations totalled 40. Of these at least three which were proved tuberculous had given negative reactions to the intradermal test, thus showing the latter to be in error to the extent of $7\frac{1}{2}$ per cent. If it be accepted, according to the subcutaneous test and examinations after slaughter, that cows No. 39, 440, 456, 476, and 478 were tuberculous, and there appears no definite reason for concluding to the contrary, then, as the intradermal test had given negative results in all these animals, the Medical Research Council's tests demonstrated that when this method was applied to cows, slaughtered immediately afterwards, its results were in error to the extent of $12\frac{1}{2}$ per cent.

(*To be continued*).

NEPHRITIS IN ANIMALS.

By Sir JOHN MCFADYEAN.

IN a text-book chapter on the subject of Nephritis one would naturally begin by endeavouring to find a basis for a scientific classification of the apparently different forms of the condition. All authors, however, appear to have found that a difficult task, mainly because in many cases the etiology of the disease is obscure and the exact sequence of the observed histological changes uncertain. There is, fortunately, one class of case that is sharply defined both by etiology and morbid anatomy and histology, *viz.*, that in which the disease results from the multiplication of pyogenic bacteria in the renal substance. Cases of this kind fall naturally into two groups : (1) Embolic or pyæmic nephritis and (2) Pyelonephritis.

EMBOLIC NEPHRITIS.

In animals, as in man, the common pyogenic staphylococci and streptococci appear to be the organisms most frequently responsible for nephritis of this type, but in the equine species, especially in the foal, a large proportion of the observed cases are caused by the *Bacillus nephritidis equi*. The term " embolic " as applied to these cases is strictly accurate when the causal bacteria arrive in the kidneys embedded in portions of thrombus material detached from the cardiac valves in acute endocarditis, but it is less exact in what appears to be the commoner cases in which the organisms have been carried free in the anterial blood. The great majority of the resulting abscesses have their starting point in the glomeruli, to which only particles of microscopic size can obtain access, and when opportunities are obtained to examine sections from animals that have succumbed at a very early stage of the kidney infection one seldom or never finds any capillaries or arterioles containing actual emboli of thrombus material. Besides, in many cases free organisms, denoting an actual septicæmia, or bacteriæmia, can be detected in the blood of the large vessels throughout the body.

It is probably safe to say that all cases of embolic nephritis are secondary to some earlier focus of disease, such as an actual abscess, an infected wound, or an acute endocarditis. These furnish, by way of the blood stream, the organisms that infect the kidneys, but between the primary lesion and the kidneys multiplication of the organisms in the blood may increase the likelihood that some of them will be arrested in the renal capillaries. In young animals particularly, multiple suppurative arthritis (joint ill) often precedes or accompanies embolic nephritis, the primary focus in these cases being at the umbilicus.

Lesions.

Macroscopic. Both kidneys are involved, and usually to the same extent. That follows from the path of the infection, and disparity can only result when some pre-existing abnormality interferes with the supply of blood to one of the kidneys. For the same reason, in each kidney the lesions are equally distributed throughout the cortex.

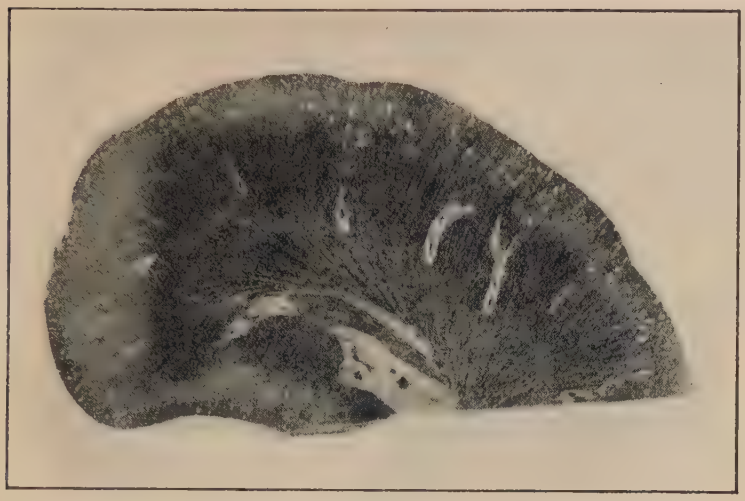

FIG. 1.

From left kidney of a Shire colt foal, five months old (two-thirds of actual size). Weight of whole organ 2 lbs. 2 ozs. Surface exposed by radial section, showing the numerous, almost evenly distributed, miliary abscesses confined to the cortex, and extreme congestion of the boundary layer and medulla. Pure culture of B. nephritidis equi obtained from the lesions.

FIG. 2.

Section of kidney of a cat (X 54). The dark bodies in the photo are masses of staphylococci. The three largest, and possibly some of the others, have had their origin in glomerular capillaries.

It is possible, even in natural cases, that sometimes only a small number of abscesses develop in the kidneys,* but, as a rule, these are practically innumerable.

The organs are swollen, often greatly, and visibly congested before section, and, unless infection has been very recent, some of the more superficial abscesses in the cortex are visible through the capsule. The latter can be detached with the greatest ease, and some times actual separation appears to have been partially effected before death.

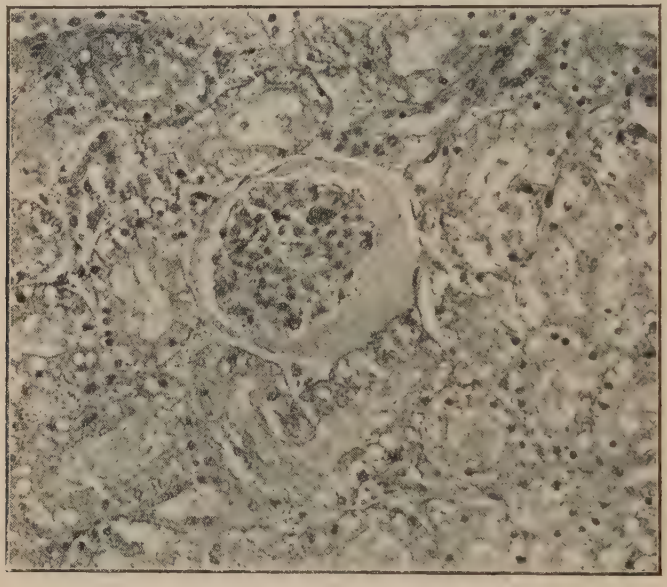

Fig. 3.

Section of kidney of a cat (X 240). From a case of streptococcus septicæmia following a suppurating wound of the leg. Abscesses were not visible to the naked eye in the kidneys. The largest dark spot in the glomerulus is a divided capillary stuffed with streptococci. The material surrounding the glomerulus was finely granular, and similar material was present in many of the convoluted tubules. Advanced fatty degeneration of the epithelium.

When a kidney is mesially divided all the strata of its substance are seen to share in the enlargement and congestion, the latter being extreme in the veins of the boundary layer. Small extravasations of blood may be visible in the cortex, and the entire kidney substance is abnormally moist.

The abscesses are usually confined to the cortex, but a few may be present in the adjacent part of the medulla. They are opaque, white or yellowish-white in colour, generally rounded in outline, and their size is usually inversely proportioned to their number—a fact explained

* The author found that in a horse infected with fully virulent sttreptococci by intravenous inoculation. The animal died from general peritonitis and pleurisy with abundant streptococci in the exudate, but only a small number of abscesses were present in the kidneys and they were of an unusually large size.

by the rapidly fatal character of the disease when the abscesses are very numerous. Very often the whole of the visible abscesses are so similar in size as to suggest a simultaneous sudden origin for the whole of them, but microscopic examination sometimes shows smaller, more recently infected, glomeruli, whose progress to macroscopic abscesses was only prevented by the animal's death.

Puncture of the abscesses with a needle reveals the liquid nature of their contents, and thus distinguishes them from other solid lesions that may to some extent simulate them.

The pelvis of the kidney is usually unaffected save for congestion.

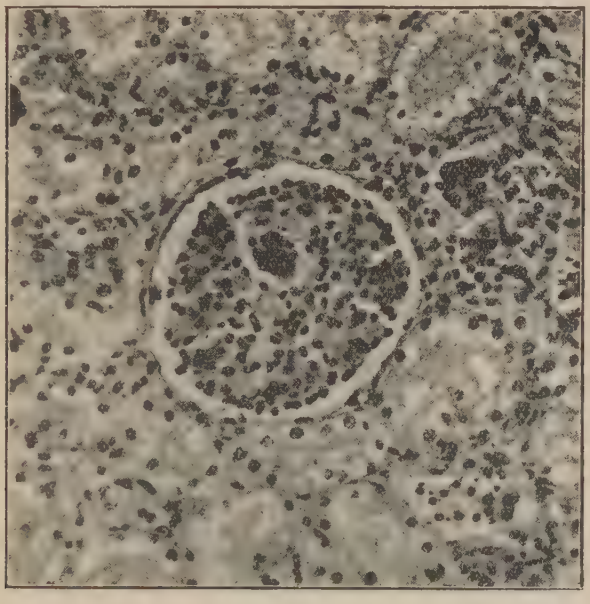

FIG. 4.

Section from kidney of foal (×250). In this case the infection appeared to be very recent. The oval dark spot above the centre of the glomerulus is composed of stained bacteria (probably B. nephritidis equi). To the right is another group of bacteria in an intertubular capillary.

Microscopic.

The mode of origin can only be recognised in very recent abscesses, as in a very short time the destructive effect of the suppuration leads to the complete disappearance of the normal renal tissue around the primary point of infection. In animals dying at an early stage, however, it is easy to recognise that the abscesses have their origin in bacteria arrested in capillaries. Glomeruli form the starting point of the great majority, but some begin in the capillaries of the intertubular plexus. The superior efficiency of the glomerular loops for the arrest of the organisms when compared with the intertubular capillaries is easily understood when it is remembered that

the main blood supply of the latter is from the efferent arterioles of the glomeruli, which, so to speak, have the first chance to stop the progress of the bacteria. It must not be inferred from this, however, that the arrest is entirely, or even mainly, of a mechanical character.

The organisms no doubt promptly begin to multiply and soon come to fill, and actually distend, the capillary at the place where the arrest took place, and this appears to be often accompanied by an extension of the growth in the length of the vessel before there is any evidence of reaction to the immediate necrotic effect of the bacteria on the capillary wall. Inspection of some of the accompanying illustrations will show how large some of these colonies of organisms may become before there is any evidence either of leucocyte emigration or of liquefaction. The absence of the one without the other is in keeping with the view that the lysin responsible for liquefaction of the tissue elements is furnished not by the bacteria, but by the leucocytes, which are predominantly of the polynuclear variety.

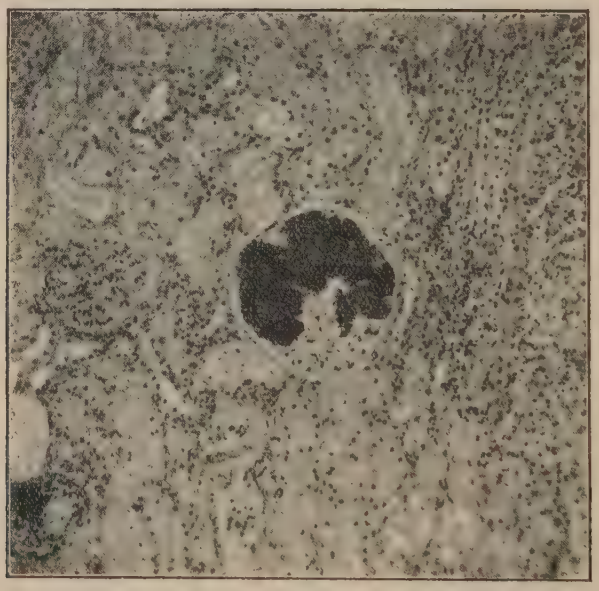

FIG. 5.

Section from kidney of a cat (× 54). Shows two-thirds of glomerular loops crammed with streptococci, the remaining capillaries in the tuft being in a state of collapse. The cat was also the subject of pericarditis, with streptococci in the exudate. The urine also contained streptococci, but mixed with B. coli.

The escape of leucocytes from the surrounding capillaries is soon followed by the complete disappearance of the tissue elements, and in this way the earlier indications of the starting point of the abscess are obliterated. Apparently the original colony of bacteria also becomes dispersed, but the individual organisms continue their multiplication, invade the renal tissue, and intensify the inflammatory action.

FIG. 6.
From same animal as Fig. 5 (× 54). Dense irregular mass of streptococci in glomerulus and afferent arteriole (on the right).

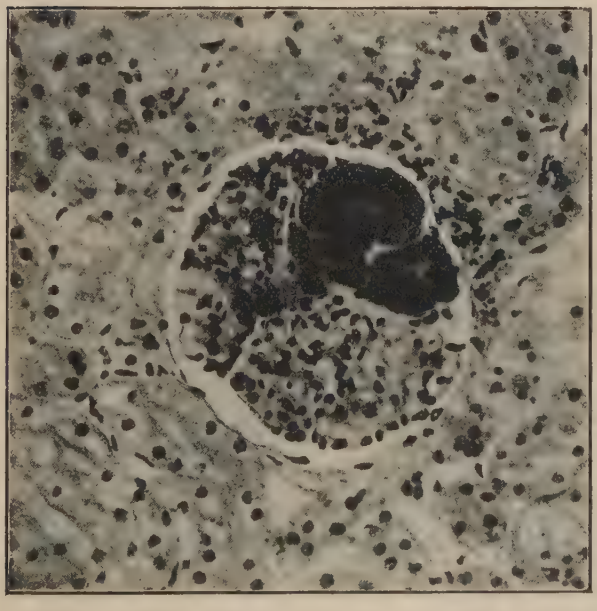

FIG. 7.
Section of kidney of a foal (× 375). The dense mass in the upper left quadrant of the glomerulus is composed of bacteria. The adjacent capillary outside Bowman's capsule is filled with polynuclear leucocytes.

Cloudy swelling, fatty degeneration, and detachment of the epithelial cells may occur throughout the convoluted tubes of the cortex, but evidence of intratubular multiplication of the bacteria is usually absent both here and in the medulla.

Clinical. Embolic nephritis is characterised by a high temperature, with all the usual signs of intense systemic disturbance, and by a reduced secretion of urine, which is blood-tinged and rich in albumen.

Death is the usual result within a day or two, and it is doubtful whether any cases recover.

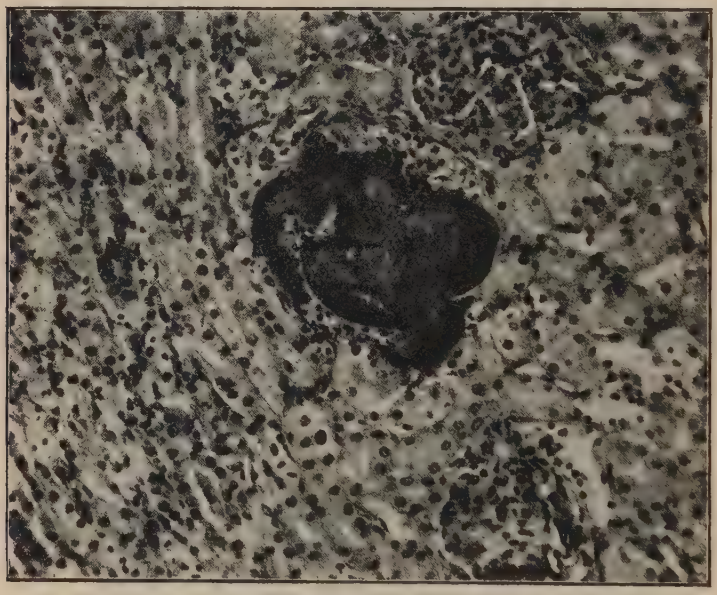

FIG. 8.

Section from kidney of a foal (× 220). Case of embolic nephritis with very small abscesses at the time of death. The dark mass of bacteria was apparently formed in intertubular capillaries.

PYELONEPHRITIS.

In cases that fall under this head the medulla and pelvis of the kidney are the main, and, at first, the exclusive, sites of the disease. Either one or both kidneys may be involved, and in the latter case the extent of the disease in the two organs is seldom the same. By these differences alone there is seldom any difficulty in distinguishing between embolic and pyelonephritis.

The literature on the subject shows that difference of opinion still exists with regard to the organisms that are most frequently responsible for the disease and the route by which they reach the kidneys. Uncertainty with regard to the first point is due mainly to the fact that post-mortem examination often reveals the presence of two or more species of bacteria in the affected parts.

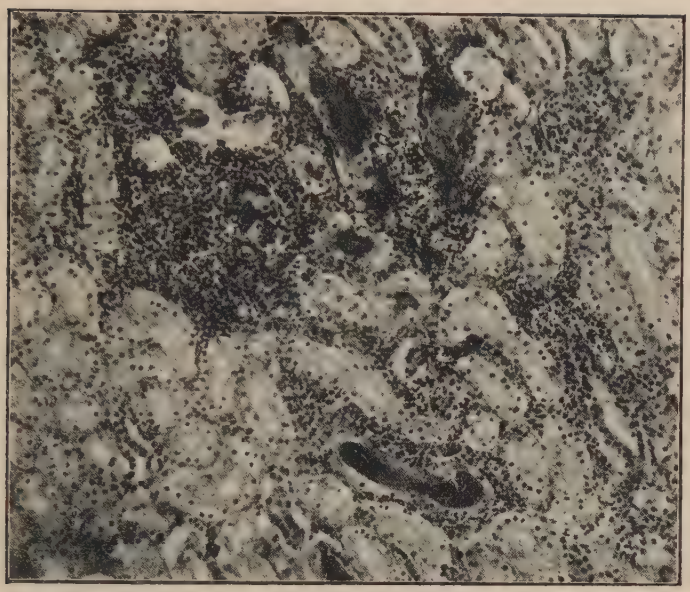

FIG. 9.

Section from cortex of kidney shown in Fig. 1 (× 220). Shows at various places masses of bacilli in renal tubules, loss of epithelium, and invasion by polynuclear leucocytes. Also in the left half of the figure a minute abscess with origin obliterated.

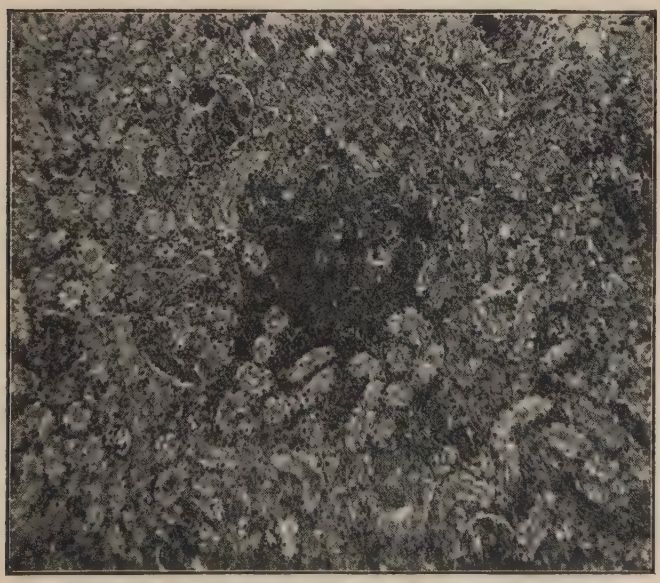

FIG. 10.

Section of kidney from same foal as Fig. 4 (× 100). Shows very small abscess, probably glomerular in origin, extending into the surrounding cortex.

The organism that appears to be the one most frequently present in the disease in cattle is *Corynebacterium renalis bovis*, so named by Ernst, who referred it to the diphtheroid group of bacteria (1905). It is probably identical with the one first cultivated in a state of purity by Höflich from six cases of the disease in cows (1891), and named by him the *Bacillus pyelonephritidis boum*.*

In other species stapylococci, streptococci, and bacilli of the B. coli type have been found in the lesions.

Clear cases of pyelonephritis occur in all the species of domesticated animals, but bovine animals provide the greatest number and, contrary to what appears to be the case in man, the condition is much commoner in the female than in the male. The great majority of the cases are in cows, and in them the disease often appears to have had its origin soon after parturition. The frequent occurrence of the disease in these animals is probably connected with the passage over the *meatus urinarius* of putrid and purulent discharges from the uterus in cases of metritis from retained afterbirth or other causes. In such circumstances the bladder readily becomes infected and the route to the kidneys is thus opened. Frequently, but not always, the source of the disease in the kidney is indicated by inflammatory changes in the bladder and ureter, although, of course, the possibility that

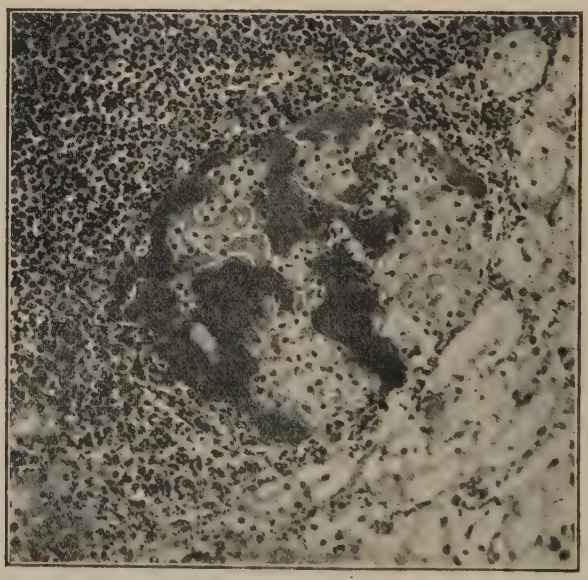

FIG. 11.

Section of kidney from same foal as Fig. 1 (× 220). Shows a glomerulus with masses of bacteria formed in its capillaries. Elsewhere its capillaries are collapsed and in part denucleated. Bowman's capsule has disappeared in consequence of advancing suppuration from an independent abscess on the left.

* For literature see Ritzenthaler, Jl. de Méd. Vét. et de Zootechnie, 1910, pp. 3 and 65.

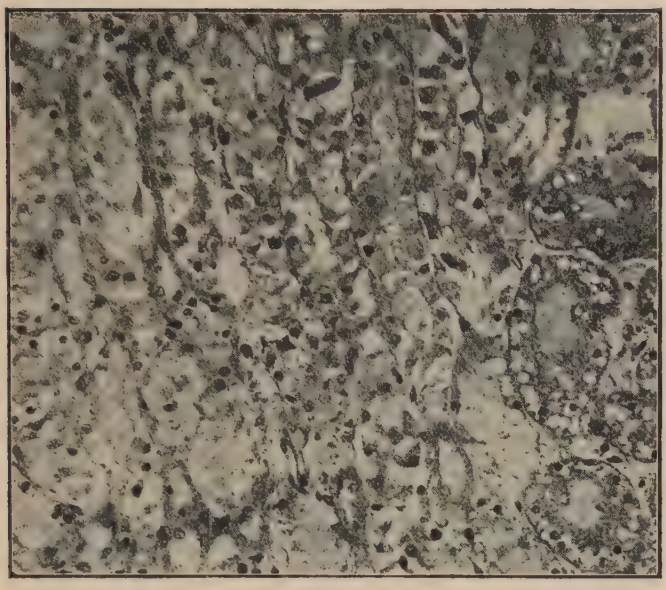

FIG. 12.

Section from the same kidney as Fig. 3 (× 200). Shows on the right margin advanced fatty degeneration of the epithelium of convoluted tubules, and elsewhere loosening and detachment of the epithelium of the tubes in a medullary ray.

FIG. 13.

Section from same kidney as Fig. 1 (× 80). The centre of the figure is occupied by a thrombus in a small vein, invaded by leucocytes on the right. It contained a large clump of bacilli in its lower half.

these may have been infected secondarily from the kidney must be admitted.

In male animals also the bladder is often involved in the inflammation, its infection with bacteria being favoured by urinary calculi or other obstacles to the free and normally frequent passage of urine.

The distribution and histology of the lesions in all but the most advanced stages of the disease admits of no room for doubt that the infection follows an ascending course, the oldest, or, it may be, the only, lesions in the kidney substance being in the medulla, and the large collecting tubes there being crammed with the bacteria.

Contrary to the view here expressed, many authors adhere to the opinion, first stated by Bollinger (1891), that the disease usually has a hæmatogenous origin.

One cannot deny as an abstract possibility that a few bacteria arrested in the kidney from the arterial blood might lead to the development of lesions simulating those found in cases of pyelonephritis, but the hæmatogenous theory is inadequate to explain the admitted facts, especially that the disease is often unilateral and that the medulla is often the main site of the lesions.

The Lesions.

Macroscopic. The lesions in different cases vary much more widely in appearance than in the embolic form, a fact explainable by the different path of invasion, the slower course of the disease, and the different organisms concerned in its causation. Sometimes, as in the embolic form, suppuration promptly follows in the invaded parts, but in others considerable necrosis precedes the formation of actual abscesses. In the case from which Fig. 14 is taken the kidney was twice the normal size but there was no liquefaction in any part, although continuous white opaque lesions extended from the medulla to the surface of many of the lobules.

At the post-mortem, the disease may be either unilateral or bilateral, but, in the latter case, as already stated, the two organs seldom or never show the uniformity in size and appearance that is the rule in embolic nephritis.

Following the infection of the kidney substance, the disease is not rapidly fatal, and time is thus often allowed for the affected organ to attain a very large size and weight (up to 8 or 9 lbs.). In extreme cases the whole of one kidney may be destroyed and be represented at the post-mortem by a mass of pus contained within the thickened capsule; at a less advanced stage the medulla may have been extensively destroyed by suppuration and lesions of a similar kind may be present in some of the lobules of the cortex. In the latter case the surface of the organ is irregular, and the capsule may be thickened and adherent at some places.

In cases obtained at an earlier stage the course of the infection in the kidney can be readily traced, since the most extensive alterations are in the medulla, and are usually associated with disease of the renal pelvis. Even when the disease has already reached the cortex, one

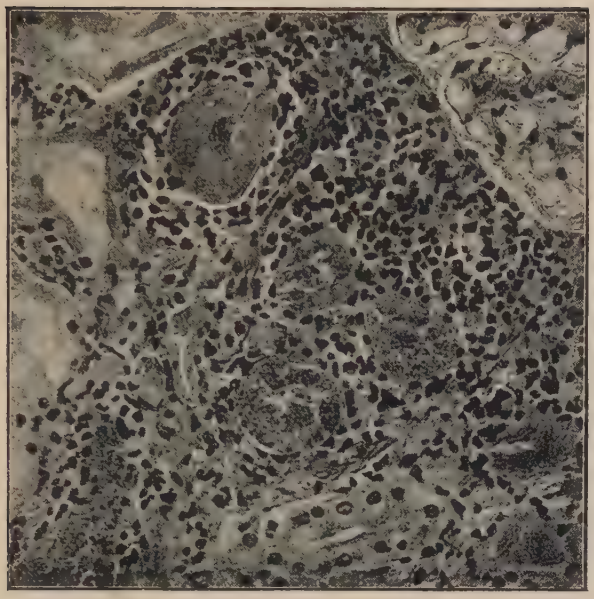

FIG. 14.

Section from cortex of the kidney in a case of pyelonephritis in a steer (× 220). Shows masses of bacilli in renal tubes whose walls have been destroyed, and dense intertubular infiltration with leucocytes. At the upper right hand is seen part of a glomerulus.

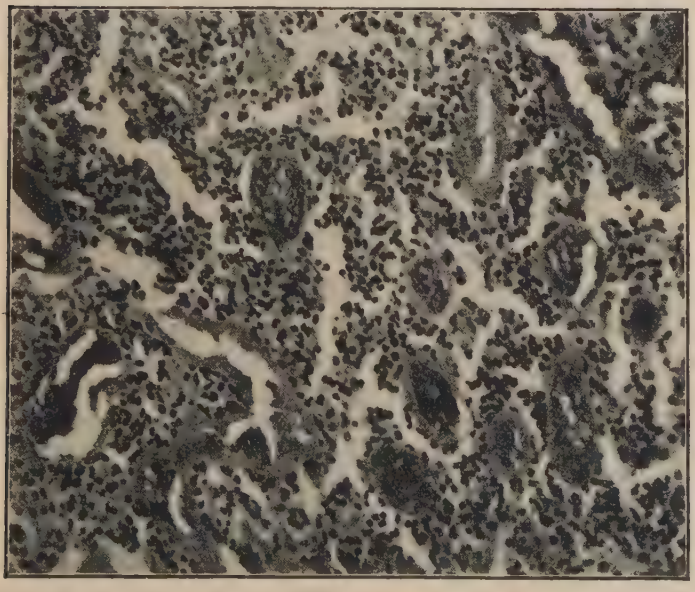

FIG. 15.

From the medulla of same kidney as Fig. 14 (× 240). All the tubes in the figures contain masses of bacilli and necrosed epithelium. Destructive intertublar infiltration with leucocytes.

may still find in the papilla of certain of the lobules opaque white or greyish streaks coinciding with the large straight uriniferous tubes up which the invasion of the bacteria is proceeding. Such areas often have on section a fan shape, attributable to the branching character of the tubes when traced upwards from the pelvis, and some of them may be directly continuous with widening areas of the same appearance in the cortex.

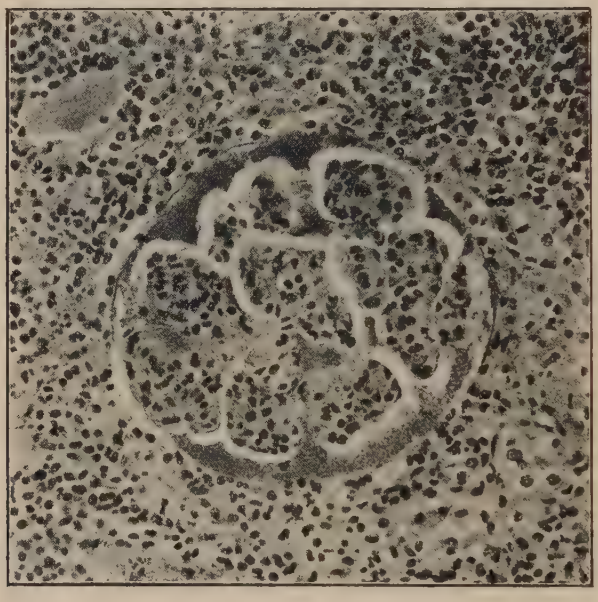

FIG. 16.

From same kidney (pyelonephritis) as Fig. 14 (× 275). The granular material between the glomerulus and Bowman's capsule (which is partially destroyed) is composed of bacilli. Some smaller masses of bacilli are also present between the lobules of the glomerulus. (For contrast with embolic nephritis, *see* Fig. 11.)

The pelvis of the diseased kidney almost invariably shows distinct alterations, varying in intensity from simple congestion to ulceration extending into the substance of the papillæ of the medulla. Contrary to what appears to be generally the case in embolic nephritis, the infection of the kidney substance in pyelonephritis does not operate simultaneously in all the lobules although all are exposed to the risk as soon as the bacteria have reached the renal pelvis. It thus happens that, even in fairly advanced cases, with extensive lesions throughout the organ, one may still find lobules of which the substance appears normal.

Microscopic. The larger abscesses show nothing special. In the progress of the suppuration the normal renal tissue has been widely destroyed and the centres at which the abscesses began have been completely obliterated. Only at the margins can the process of

destruction be followed, where the infiltration with polynuclear leucocytes causes the disappearance of the tubes and other elements of the original tissue of the kidney as if by a process of digestion.

In kidneys obtained at an early stage one can follow the invasion of the large tubes of the medulla by the bacteria. The immediate effect is degeneration and death of the epithalial cells followed by the leucocyte invasion from the neighbouring capillaries, which are usually intensely congested.

At no stage does one find evidence that the abscesses are started by the arrest of bacteria in glomerular or other capillaries. In the cortex, as in the medulla, the invasion appears to be mainly by way of the urinferous tubes, and it is also by this route that the bacteria invade the cortex. In the course of this invasion bacteria occasionally penetrate into Bowman's capsule and multiply there, even penetrating between the loops of the glomerulus. Such an occurrence is easily distinguished from the arrest of bacteria in the glomerular capillaries in embolic nephritis (compare Figs 11 and 16).

Clinical. Bollinger thought that pyelonephritis was the third most frequent disease of cattle in Germany, and, although that opinion would hardly be confirmed to-day, it appears to be of common occurrence in cows everywhere.

The course of the disease is usually sub-acute or chronic. The urine is more or less turbid, sometimes ammonical in odour, and contains a considerable or large amount of albumen.

On standing it yields a copious deposit of pus and other cells, granular matter, bacteria, and triplephosphate crystals. When the bladder is involved a large proportion of the suspended materials may come from that organ.

(*To be continued.*)

REVIEWS.

Minerals in Pastures and their relation to Animal Nutrition. By J. B. Orr, D.Sc., M.D., with the assistance of Helen Scherbatoff. From the Reid Library, Rowett Research Institute, Aberdeen. London: H. K. Lewis & Co., Ltd., 1929. Pages 150, Royal 8vo. Price 10s. 6d.

This work embodies the results of an endeavour to ascertain, by a search of the literature on the subject and a questionnaire addressed to the various Governments within the Empire, to what extent deficiency of minerals in pastures was the cause of malnutrition in cattle and sheep. The earlier chapters are devoted to a general consideration of the mineral content of pastures and the factors affecting the same, including the species and stage of growth of plants, the climatic conditions, and the nature of the soil. The bulk of the book sets out what has been ascertained regarding the occurrence of "deficiency diseases" in cattle and sheep in different parts of the world, and a special chapter is allotted to such diseases in horses. As the work shows, the loss inflicted by deficiency diseases does not constitute the whole of the evil that may be caused by feeding animals on grass or hay with a defective mineral content, for the presence of the essential minerals in adequate amount is an important factor in determining the nutritive value of the pasture. It is a noteworthy fact that in the comprehensive survey which the work gives of the occurrence of deficiency diseases throughout the world, there is very little evidence that they are of serious economic importance in Great Britain, with the possible exception of certain tracts of mountain pasture in Scotland. An example of evidence that needs confirmation is the statement (p. 109) that the administration of potassium iodide to mares prevented joint-ill in foals in a stud in the South-west of Scotland.

Veterinary surgeons in rural practices would be well advised to buy the book.

Welche Klimafaktoren sind für den Asthmatiker von Wichtigkeit? Von Dr. Viktor Grimm. Berlin: Richard Schoetz, 1928. Pages 284, 8vo. Price, 12·60 marks.

The author of this work has done good service in making public what he has been able to learn regarding the etiology of asthma from a study of the existing literature, his own large experience as a physician at the Asthmabad, Reichenhall, Bavaria, and information supplied to him in response to a questionnaire addressed to the members of the medical profession in Holland. Its appearance is opportune in view of the National Campaign against the disease that has recently been initiated in this country. All the known or suspected causes of asthma are dealt with, and special interest attaches to the part that deals with those cases in which the excitant is derived from horses, fowls, and other domesticated animals and records many proved instances.

Das Lymphgefässystem des Pferdes. Von Dr. Herman Baum. Berlin: Julius Springer, 1928. Price, 38 marks.

This work, which has 135 Royal quarto pages of print and 53 illustrations (with the lymph vessels in colour), constitutes a most valuable addition to the literature of Veterinary Anatomy. It does for the lymph vessels of the horse what had previously been done by the same distinguished author for these vessels in the ox and dog. It reflects great credit alike on the author, the artists who collaborated with him, and the publishers.

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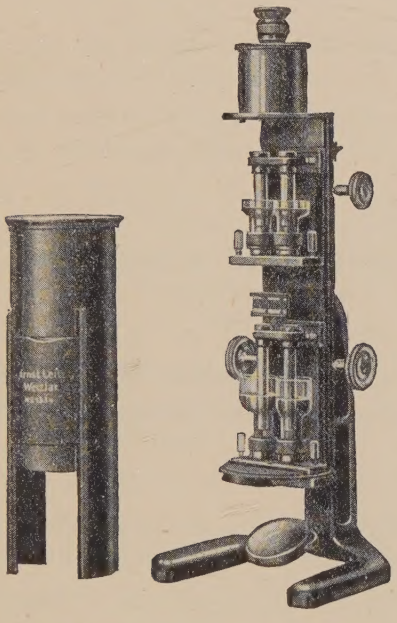

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